The association between tuberculosis and the development of insulin resistance in adults with pulmonary tuberculosis in the Western Sub-District of the Cape Metropole region

by

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work and that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

Introduction: The existence of a bi-directional relationship between tuberculosis (TB) and insulin resistance (IR)/diabetes has been alluded to in literature. Although diabetes has been linked to increased TB risk, the relationship between TB as a causative factor for IR remains unclear. The study aimed to determine if an association existed between TB and IR development in adults with newly diagnosed pulmonary tuberculosis (PTB) at baseline. It was additionally aimed to document changes in IR status during follow-up.

Methods: This observational, cross-sectional study evaluated ambulatory participants at baseline for IR prevalence via anthropometrical and biochemical measures, together with diagnostic IR tests [homeostasis model assessment–IR (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI)]. In addition, a prospective cohort sub-section study was performed on approximately half of the baseline study population (n=29), who were followed-up at two and five months whilst on TB treatment. Summary statistics, correlation co-efficients and appropriate analysis of variance were used to describe and analyse data. Participants were excluded if they presented with other forms of TB, were HIV-positive, obese or had any pre-disposing IR conditions such as diabetes or metabolic syndrome.

Results: A total of 59 participants were included from August 2013 until December 2014. The majority of participants were male (81.4%) and the mean age was 33.95 ±12.02 years. The prevalence of IR was 25.4% at baseline, determined by using a calculated HOMA-IR cut-off point of 2.477. Patients with IR were shown to be younger ($p=0.04$) and had a higher fasting insulin level ($p<0.01$). Although the difference between IR levels in participants between baseline and follow-up was not significant, a decrease was experienced over time.

Most participants (61.0%) presented with a normal BMI at baseline and the majority of anthropometrical measurements showed a significant increase over the follow-up period, mainly in the first two months of treatment.

The majority of participants (84.7%) had an increased mean CRP (60.18 ±50.92 mg/L) and decreased mean HDL-cholesterol level [69.5% (males: 0.94 ±0.88 mmol/L; females: 1.14 ±0.88 mmol/L)] at baseline. Mean baseline values of fasting glucose and albumin were within normal ranges (4.82 ±0.80 mmol/L and 39.32 ±4.35 g/L respectively). According to fasting glucose levels at baseline, 1.7% and 3.4% of participants presented with impaired fasting glucose and diabetes

mellitus respectively. Several biochemical markers (CRP, albumin and white cell count) showed an improvement during the follow-up period.

Conclusion: The study found an association between TB and IR development in newly diagnosed PTB patients. Many anthropometrical and biochemical measures showed improvements with time, especially during the intensive phase of treatment. Although not significant, IR levels decreased over time, which could be indicative of a clinical improvement. IR participants were shown to be younger and had a higher fasting insulin measurement. A high prevalence of IR among TB patients therefore highlights the need for early identification in order to facilitate a reversal of IR and prevent possible IR-related complications.

ABSTRAK / OPSOMMING

Inleiding: Die tweerigtingverhouding tussen tuberkulose (TB) en insulienweerstandigheid (IW)/diabetes is al in literatuur beskryf. Diabetes hou wel verband met ’n verhoogde risiko vir TB, maar die rol van TB as oorsakende faktor vir IW bly onduidelik. Die doel van hierdie studie was om te bepaal of daar wel ’n verband bestaan tussen TB en die ontwikkeling van IW onder volwassenes met nuut gediagnoseerde pulmonale tuberkulose (PTB). ’n Addisionele doel was om enige veranderinge in die IW status, tydens opvolg ondersoeke, te dokumenteer.

Metodes: Die waarneming, deursnee-studie het ambulante deelnemers tydens die basislyn periode geëvalueer vir IW d.m.v antropometriese en biochemiese bepalings, asook diagnostiese IW toetse [“homeostasis model assessment–insulin resistance” (HOMA-IR) en die “quantitative insulin sensitivity check index” (QUICKI)]. ‘n Prospektiewe kohort sub-gedeelte studie is ook uitgevoer op ongeveer die helfte van die basislyn studiepopulasie (n=29), wat op twee en vyf maande opgevolg was tydens TB behandeling. Beskrywende statistiek, korrelasie koeffisiënte en toepaslike analises van variansie is gebruik om data te beskryf en analiseer.Deelnemers is uitgesluit indien hulle met enige ander vorm van TB gediagnoseer is, MIV-positief of vetsugtig was of presenteer het met enige predisponerende IW toestande, soos diabetes of metaboliese sindroom.

Resultate: Nege en vyftig deelnemers is ingesluit tussen Augustus 2013 en Desember 2014. Die meerderheid was manlik (81.4%) met ’n gemiddelde ouderdom van 33.95 ±12.02 jaar. Die basislyn prevalense van IW was 25.4%, bepaal deur ’n berekende HOMA-IR afsnypunt van 2.477. IW pasiënte was jonger (p=0.04) en besit ’n hoër vastende insulienvlak (p<0.01). Alhoewel daar geen beduidende verskil in die IW vlakke tussen basislyn en opvolg periodes was nie, was daar wel ’n afname waargeneem oor tyd.

Die meeste deelnemers (61.0%) het ’n normale LMI by basislyn gehad. Die meerderheid antropometriese metings het ’n beduidende toename getoon tydens die opvolg periode, hoofsaaklik in die eerste twee maande van behandeling.

Die meerderheid deelnemers (84.7%) het ’n verhoogde gemiddelde CRP (60.18 ±50.92 mg/L) en ’n verlaagde gemiddelde HDL-cholesterolvlak [69.5% (mans: 0.94 ±0.88 mmol/L; vrouens: 1.14 ±0.88 mmol/L)] by basislyn getoon. Die gemiddelde basislyn waardes van vastende glukose en albumien was binne normale perke (4.82 ±0.80 mmol/L en 39.32 ±4.35 g/L onderskeidelik). Volgens die basislyn vastende glukosevlakke, is 1.7% met ingekorte vastende glukose en 3.4% met diabetes

mellitus geklassifiseer. Verskeie biochemiese aanduiders (CRP, albumien en witsel-telling) het ’n verbetering tydens die opvolg periode getoon.

Gevolgtrekking: Hierdie studie het ‘n assosiasie tussen TB en IW-ontwikkeling, in nuut gediagnosserde PTB pasiënte, getoon. Verskeie antropometriese en biochemiese metings het verbeter met tyd, veral tydens die intensiewe fase van behandeling. Alhoewel nie beduidend, het IW vlakke met tyd afgeneem, wat moontlik ’n kliniese verbetering aandui. Deelnemers met IW was jonger en het hoër vastende insulien vlakke getoon. Hoë voorkoms van IW onder TB pasiënte beklemton die behoefte vir vroeë identifisering, om sodoende, ’n omkering in IW te fasiliteer en IW-verwante komplikasies, waar moontlik, te voorkom.

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CONTRIBUTIONS BY PRINCIPAL RESEARCHER AND FELLOW RESEARCHERS

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AACE	American Association of Clinical Endocrinologists
ABC	ATP-binding cassette transporter-1
ADA	adenosine deaminase
AFA	arm fat area
AFB	acid fast bacilli
AIDS	Acquired Immunodeficiency Syndrome
AMA	arm muscle area
ANACOVA	appropriate analysis of covariance
ANOVA	appropriate analysis of variance
ApoB	apolipoprotein B
APR	acute phase response
ARDS	acute respiratory distress syndrome
ART	antiretroviral therapy
ATP III	Adult Treatment Panel III
AUC	area under curve
BCG	bromocresol green solution
BIA	bioelectrical impedance analysis
BMI	body mass index
BP	blood pressure
CIGMA	continuous infusion of glucose with model assessment
CMI	cell mediated immunity
CoCT	City of Cape Town
CRP	C-reactive protein
CT	computerized tomography
CVD	cardiovascular disease
CXR	chest x-ray
D	body density

DECODE	Diabetes Endocrinology: Collaborative Analysis of Diagnostic Criteria in Europe
DEXA	dual energy x-ray absorptiometry
DM	diabetes mellitus
DNA	deoxyribonucleic acid
DoH	Department of Health
EGIR	European Group for the study of insulin resistance
EPTB	extra-pulmonary tuberculosis
EQA	external quality assessment
ESR	erythrocyte sedimentation rate
FFA	free fatty acids
FFM	fat free mass
FIRI	fasting insulin resistance index
FM	fat mass
FSIVGTT	frequently sampled intravenous glucose tolerance test
GI	glucose insulin
GXP	gene Xpert
HbA1c	glycosylated haemoglobin
HBC's	high burden countries
HCT	HIV counselling and testing
HDL	high density lipoprotein
HEC	hyperinsulinaemic euglycaemic clamp
HIV	Human Immunodeficiency Virus
HOMA	homeostasis model assessment
HOMA-IR	homeostasis model assessment – insulin resistance
HPT	Hypertension
HREC	Health Research Ethics Committee
ICD-10	International Statistics of Diseases and Health Problems – 10th revision

IDF	International Diabetes Federation
IFG	impaired fasting glucose
IFN-γ	interferon-gamma
IGF-1	insulin-like growth factor – 1
IGFBP-1	insulin growth factor binding protein – 1
IGI	insulinogenic index
IGRA	interferon gamma release assays
IGT	impaired glucose tolerance
IL-1β	interleukin-1β
IL-1ra	interleukin-1ra
IL-4	interleukin-4
IL-6	interleukin-6
IL-10	interleukin-10
IMT	intima-media thickness
INH	Isoniazid
IR	insulin resistance
IRAS	Insulin Resistance and Atherosclerosis Study
IST	insulin infusion sensitivity test
ITT	insulin tolerance test
LAM	lateral flow version
LBM	lean body mass
LDL	low density lipoprotein
LED	light emitting diode
LPA	line probe assay
MCP-1	macrophage chemo-attractant protein-1
MDGs	Millennium Development Goals
MDR-TB	multi-drug resistant tuberculosis
MINMOD	minimal model analysis
MRC	Medical Research Council

MRI	magnetic resonance imaging
m-RNA	messenger ribonucleic acid
MSE	mean square error
MTB	mycobacterium tuberculosis
MUAC	mid-upper arm circumference
NAT2	N-acetyltransferase 2
NCEP ATP III	National Cholesterol Education Program – Third Adult Treatment Panel
NEFA	non-esterified fatty acids
NHLS	National Health Laboratory Services
NIDDM	non-insulin dependent diabetes mellitus
NRF	National Research Foundation
NTP	Nutritional Therapeutic Programme
OGIS	oral glucose insulin sensitivity
OGTT	oral glucose tolerance test
PAI-1	plasminogen activator inhibitor-1
PCOS	polycystic ovarian syndrome
PCR	polymerase chain reaction
PEM	protein energy malnutrition
PTB	pulmonary tuberculosis
QUICKI	quantitative insulin sensitivity check index
RD	registered dietician
RIF	Rifampicin
RMANOVA	appropriate repeated measured analysis of variance
RMSSE	root mean square standardized effect
ROC	receiver operating characteristic
ROS	reactive oxygen species
R-QUICKI	revised-QUICKI
SA	South Africa
SAA	serum amyloid

SANAS	South African National Accreditation System
SANHANES-1	South African National Health and Nutrition Examination Survey
sCD36	soluble CD 36
SD	standard deviation
SDGs	Sustainable Development Goals
SEMSDA	Society for Endocrinology, Metabolism and Diabetes in South Africa
SHBG	sex hormone-binding globulin
sPLA2	secretory phospholipase A2
SU	Stellenbosch University
SuRFNCD-2007	3rd National Surveillance of Risk Factors of Non-communicable Diseases
TAH	Tygerberg Academic Hospital
TAT	turn around time
TB	Tuberculosis
TGF-β	transforming growth factor – beta
TNF-α	tumour necrosis factor – alpha
US	United States
VAT	visceral adipose tissue
VD	volume of distribution
WC	waist circumference
WCC	white cell count
WHO	World Health Organization
WHR	waist:hip ratio
XDR-TB	extensively drug resistant – tuberculosis
ZN	Ziel-Neelson

DEFINITION OF TERMS	
Acute phase response	A number of changes (including behavioural, physiological, biochemical and nutritional) occurring during the first days following an insult to the body.[1]
Anthropometry	The science of measuring the size, weight and proportions of the human body.[2]
Biochemistry	Tests available for assessing the nutritional status of an individual. These tests can be either static or functional.[2] Static tests (undertaken in this study) relate to the measurement of a specific nutrient (or its metabolite) in blood, urine or bodily tissues.[2]
Diabetes mellitus	A metabolic disorder with varying aetiologies, which is recognised for its long-term hyperglycaemia and alterations in macronutrient (carbohydrate, fat and protein) metabolism, which are mainly resultant from defective insulin secretion, action or a combination of the two.[3]
Extra-pulmonary tuberculosis	Forms of tuberculosis occurring when mycobacteria migrate from the lungs to other organs and joints or central nervous system and cause disease. Usually these bacilli will be controlled by the body's immune system and disease will not arise.[4,5]
Fasting insulin resistance tests	Tests that are based on fasting blood values.
Group 1	Participants in the total study population that were seen only at baseline
Group 2	Participants in the total study population that were seen for a total of three visits (namely baseline, 2 and five months)

HOMA-IR	A fasting index used in the determination of basal insulin resistance,[6] using only the fasting insulin and glucose values.[7]
Insulin resistance	A state in the body whereby cells become increasingly resistant to insulin, in which increased insulin levels are necessary to elicit a normal physiological response.[8-12]
Intensive phase	The initial two months after treatment commencement, when a more aggressive regimen of TB drugs is prescribed, namely isoniazid, rifampicin, ethambutol and pyrazinamide.[5]
Invasive insulin resistance tests	Tests that require intravenous insulin/glucose infusions.[13]
Metabolic syndrome	A condition encompassing the following occurrences: abdominal obesity, atherogenic dyslipidaemia, elevated blood pressure, insulin resistance, and/or a pro-inflammatory/thrombotic state.[14-17]
Non-invasive insulin resistance tests	Measurements that do not require the administration of either exogenous insulin or glucose via the intravenous route.[13]
Primary infection	Time of first exposure to bacilli in a previously unexposed individual.[5]
Pulmonary tuberculosis	*Mycobacterium tuberculosis* infection occurring in the lungs, which is also the most infectious and widely recognised form.[8] Tuberculosis (general term) is regarded as a significant airborne-transmitted bacterial infection, resulting from infection with *M tuberculosis* bacilli.[18,19]
QUICKI	Another example of a fasting index for IR. Inverse logarithm of the HOMA-IR, also utilizing fasting insulin and glucose measurements.[20]
Secondary infection	Period of disease occurring after the primary infection.[21]

CHAPTER 1: REVIEW OF THE LITERATURE

1.1 INTRODUCTION

The aim of this study is to generate new information regarding tuberculosis (TB) and the possible development of insulin resistance (IR) in a typical South African setting, which is a subject that has not received much attention thus far. This literature review describes the current TB epidemic, on both a global and national scale, and attempts to explore the important facets of TB transmission, infection, clinical presentation and diagnosis, among others. An important component of the literature review is also dedicated to the impact of TB on nutritional status, where aspects such as malnutrition among TB patients, changes in anthropometrics and micronutrient status, as well as various biochemical changes occurring during the course of the disease, are analysed.

The concept of IR is examined, and this includes the aetiology, prevalence rate, as well as a discussion on the metabolic syndrome that often follows an insulin resistant diagnosis. A crucial section on the current methods available for measuring IR is also included, detailing known strengths and limitations of each method. The review concludes with an up-to-date presentation of current evidence regarding the bi-directional relationship between IR and/or diabetes mellitus (DM) and TB and provides information on the possible mechanisms of action.

Since the subject matter on hand encompasses such a broad spectrum, it must be noted that the literature review is selective in terms of discussion points, because many of the aspects, although of interest, are beyond the scope of this particular review.

1.2 TUBERCULOSIS

1.2.1 Background – A Problem on a Global and National Scale

1.2.1.1 Global overview

Tuberculosis is commonly regarded as an extremely significant bacterial infection, affecting a large proportion of the global population.[19] It is reported to be the second leading cause of mortality due to infectious disease worldwide, following the Human Immunodeficiency Virus (HIV) / Acquired Immune Deficiency Syndrome (AIDS).[18,22] This led the World Health Organization (WHO) to declare TB a global public health emergency in 1993. Statistics released by the WHO in 2014, show that there were an estimated nine million cases of TB diagnosed globally in 2013.[18]

Demographic data shows that men are more likely to be infected with TB than women.[18,23] Global reports indicate approximately 60% of infections and deaths are found among male patients. Furthermore, two-thirds of infections are found to occur in the 15 to 59-year-old age group,[18] although the young adult group seems to be the most affected.[23] It is thus evident that the nature of the disease is such that it strikes at the heart of the economic work force, most often in developing, third-world countries, of which South Africa (SA) is one.

Of the 9 million individuals who developed TB in the past year, approximately 1.1 million were found to be HIV-positive.[18] If one considers statistics from South Africa regarding the HIV-positive rate among TB patients, it ranks significantly higher than the global percentage.[24] This is in line with the findings that show South Africa to have a very high disease burden of both HIV and TB.

When comparing statistics of incident TB cases, between the six WHO global regions (African Region, Region of the America's, Eastern Mediterranean Region, European Region, South-East Asia Region and Western Pacific Region), the majority (56%) were found in the South-East Asia and the Western Pacific regions. The African Region was responsible for 25% of cases, and was also found to have the highest rate of cases and mortality in relation to population size.[18,23] It has also been shown that the African Region is responsible for approximately 80% of HIV-positive TB cases among HIV-infected individuals.[18] If one considers the rates of infection in the African Region, there is an improvement, but this is unfortunately still slower than what is required for successful TB control.

Global mortality rates as a result of TB disease indicate that approximately 1.5 million people died from the condition in 2013.[18] Of these 1.5 million deaths, 360 000 were found to be living with HIV (24%), which is line with TB being a common opportunistic infection associated with HIV/AIDS. Tuberculosis has been recognised as the principal cause of HIV-related mortality despite being a treatable condition.[25] Another disillusioning statistic shows that approximately 78% of the total TB deaths occurred in the African and South-East Asian regions, again showing the vulnerability of the African Region.[18]

Despite these dire statistics, it was found that global TB rates are on the decline (the prevalence rate has decreased globally by 41% since 1990),[18] which is partly due to the efficacious diagnosis and treatment of infectious individuals. This may not seem accurate if one considers the higher number of recorded incident cases and mortalities compared with previous years, but this is in fact attributable to improved recording and reporting methods and not to a greater number of TB cases. Furthermore, promising global treatment success rates in 2013 showed as many as 86% of cases being effectively treated.[18] Another promising factor is that, despite Africa's high percentage of HIV-

infected individuals, 76% of these persons had been tested for HIV and were aware of their status in 2013.[18]

Tuberculosis and its subsequent eradication remains one of the key priorities for global health organisations. The Global Plan to Stop TB 2006–2015 was initiated in 2005 by the Stop TB Partnership, an organisational network that hopes to both curb the spread of and eliminate TB as a public health problem.[22,26] This target and concomitant timeline tie in closely with the Millennium Development Goals (MDGs), specifically MDG 6, "Combat HIV/AIDS, malaria and other diseases" by the year 2015,[27,28] which also aims to reduce global TB incidence rates.[18] Since this target date has already transpired, a new set of attainable targets has been devised and is being considered for approval. The new goals, aptly named the Sustainable Development Goals (SDGs),[29,30] are a list of 17 objectives with a target deadline of 2030. These goals have not formally been accepted as a framework but are expected to be finalised towards the end of 2015. One of these new goals relates specifically to the issue of TB, namely, "to ensure healthy lives and promote well-being for all at all ages." [29] This is similar to the previous MDG; the new goals include a target to "end the epidemics of AIDS, TB, malaria and neglected tropical diseases and combat hepatitis, waterborne diseases and other communicable diseases".[18]

1.2.1.2 National overview

2013 statistics show that South Africa is considered by the WHO to be one of twenty-two "high burden countries" (HBC's).[18,31,32] These HBC's are duly named because they house approximately 80% of the world's TB cases. Other African HBC's include the Democratic Republic of the Congo, Ethiopia, Kenya, Mozambique, Nigeria, Uganda, the United Republic of Tanzania and Zimbabwe. It was largely due to this non-prestigious classification that the South African Department of Health (DoH) declared TB to be at an official crisis-level, and this resulted in the compilation of a TB national crisis management plan, which was released in 2006.[33]

Assessment of South Africa's progress in relation to achieving the targets set out by the MDGs indicate that whilst SA has achieved a declining TB incidence rate, other markers have not been so readily achieved. These include halving the TB mortality rate, halving the 1990 TB prevalence level and meeting the 2015 targets for lowering TB cases and mortality rates. South Africa is by far not the worst performing HBC, but it is clear that progress is still needed in eradicating this disease.

1.2.1.3 Provincial overview

According to 2013 statistics specific to the Western Cape, there were 183 628 suspected TB cases identified in 2013 for the entire province. Of these, 183 512 persons were tested (99.93%), which is a promising screening rate. A total of 19 893 individuals were identified as being sputum smear-positive with TB (10.8%). The total number of outpatients in attendance for more than five years at various facilities around the province amounted to 12 157 911 individuals.[34]

Data extrapolated from the Cape Metropole of the Western Cape show the following statistics for the Western Sub-District of the region. Data gathered in the first three-quarters of 2014 show an average of 624 TB suspects per month (a suspect is an individual who is suspected of having active TB disease on the grounds of physical signs or symptoms that are synonymous with TB, such as a persistent cough).[4,35] Of these suspects, 52.9% were male and 47.1% were female. Of the number of individuals actively diagnosed with TB disease, approximately 216 new cases per month in the Western Sub-District were identified, with pulmonary tuberculosis (PTB) accounting for 88.1% of cases. Of these newly identified TB cases, 51.0% (n=991) were shown to be HIV-negative, whilst HIV-positive and those with unknown status accounted for the remainder of the population.[36]

Upon examining data generated from the Albow Gardens clinic (the recruitment site for this study), the following data for the first three-quarters of 2014 was noted. Newly diagnosed and registered TB patients accounted for an average of 28 new patients per month, of which the majority (79.7%) were diagnosed with PTB. The majority (52.5%) of cases treated by the clinic were also HIV-positive. Of the newly registered patients, 80.1% were cases of first-time TB and 19.9% were re-treatments.[36]

It is, therefore, evident that the population of the Western Cape is extremely susceptible to the effects of TB, and the area is thus one of priority.

1.2.2 Transmission of TB

Tuberculosis results from infection with the bacillus *M tuberculosis,* of which the primary target site of the bacteria is the lungs.[18] This is typically referred to as PTB, but the infection can also occur in other areas of the body such as the abdominal cavity and brain.[8] The spread of the disease is classed as airborne and is distributed when infectious persons release infectious droplets containing a small amount of *M tuberculosis* bacilli into the air, via coughing, spitting, sneezing, singing or talking.[18,37] The transmission of TB is dependent upon three factors, namely the number of organisms released into the air, the concentration of said organisms and the length of time an exposed person is in

contact with the contaminated air.[5] These bacteria fare better in dark areas because exposure to direct sunlight results in quick death of the bacilli.

The majority of people who are infected with the TB bacilli remain asymptomatic, and the estimation by the WHO is that although approximately one-third of the population are latent carriers, only 10% will progress to clinical TB, with 50% of these individuals developing active disease at the time of infection and 50% at a later stage.[5,18,19,37] This estimation is, however, based on healthy individuals, whereas there is an increased risk of developing active TB if an individual becomes immuno-compromised.[22] In addition, other vulnerable groups for infection include children, HIV-infected individuals and the elderly.[5] Immune-compromised conditions such as diabetes mellitus, silicosis and prolonged use of corticosteroid agents and other immune-suppressants have also been linked to faster progression to active TB.[5]

Part of the problem experienced through the global TB epidemic is the slow progression of the disease, which allows persons to present asymptomatically and not seek treatment. This delay in commencing suitable medication places others in the population at risk because an untreated person with active TB disease can infect as many as 15 unsuspecting persons.[38]

A systematic review conducted in 2014 concluded that aspects placing an individual at risk of TB included low-income households, poor educational opportunities, sub-optimal living conditions (overcrowding), substance abuse (alcohol, drugs and smoking), pre-existing conditions such as malnutrition and diabetes, indigenous communities and migration, lack of accessibility to appropriate health care and inadequate knowledge regarding TB. The majority of these are rife in a developing country such as South Africa.[39] In addition, HIV infection and indoor air pollution were also identified to be prominent risk factors in the spread of the disease.[38]

1.2.3 Primary and Secondary Infection

1.2.3.1 Primary infection

The infection occurs at the time of first exposure to TB bacilli of a previously unexposed individual.[5] The infectious droplets are inhaled into an alveolus and the bacilli are phagocytosed by alveolar macrophages. [21,40] Bacterial multiplication and growth within the macrophage then occurs, and this consequently generates an inflammatory response in the carrier. This stage of infection is not usually accompanied by any symptoms and is thus difficult to diagnose, apart from a positive tuberculin skin test 4 - 6 weeks after the infection occurred.[5,21] The risk of developing active disease is highest during the first two years after primary infection.[21]

1.2.3.2 Secondary (or post-primary) infection

This refers to the period of the disease occurring after the primary infection, i.e. in a previously infected human host and is often known as 'reactivation'.[21] There is no pre-determined time period for the development of active disease because progression can be attributed either to re-infection with *M tuberculosis* or to reactivation of previously dormant bacteria.[5] This post infection commonly affects the lungs, but the infection can spread to other parts of the body and may cause disease. This occurs most commonly after a primary infection, but may also be seen in HIV-infected individuals.

1.2.4 Different Types of TB

Pulmonary tuberculosis is the most common and widely recognised form of TB disease. It is also the most infectious and represents the majority (80%) of cases among TB-infected persons.[5] However, HIV-infected individuals can often exhibit extra-pulmonary forms of the disease.[21] Other forms of TB commonly occur when mycobacteria migrate from the lungs to other organs, and this is commonly referred to as extra-pulmonary tuberculosis (EPTB). Extra-pulmonary tuberculosis occurs in many forms, of which the most common types include TB lymphadenitis, TB of the bones and joints, TB of the spine or central nervous system, TB meningitis, disseminated/miliary TB effusion (pleural, pericardial and peritoneal) and tuberculous empyema.[4,5] The miliary form of the disease often presents concomitantly with severe symptoms such as septic shock and acute respiratory distress syndrome (ARDS), which can increase mortality rates.[41-43]

1.2.5 Clinical Presentation of PTB

Symptoms associated with PTB are very often wide-ranging and are not always indicative of TB since they could be caused by other co-morbidities or infections. Symptoms include:

- Chronic, persistent cough for two weeks or more
    - HIV-positive patients presenting with a cough (even if less than two weeks) should be identified
    - Occasionally blood-stained sputum
- Fever of more than two weeks duration
- Drenching night sweats
- Unintentional/unexplained weight loss
    - Usually more than 1.5 kg in a month
- Chest pains / breathlessness / localised wheeze
- Fatigue
- Anorexia[5,21,44,45]

A patient with TB is therefore classified as bacteriologically confirmed TB or as having commenced TB treatment by a health care worker based on the patient's clinical presentation, X-ray findings or other diagnostic tests.[5]

1.2.6 Diagnosis of TB – Different Methods/Tools

1.2.6.1 General

As mentioned previously, a worldwide decline in incident TB rates has largely been due to effective diagnosis and treatment of the disease and improved socio-economic conditions. However, diagnosis still poses problems that need to be carefully managed by health workers in contact with suspected individuals. Furthermore, effective diagnosis and management is directly related to persons with TB-like symptoms going for check-ups at their closest health facility, the screening for TB in health care facilities and the wider community, the reliability and validity of diagnostic tests, the turnaround time for laboratory testing, as well as the notification of individuals of their positive status and the commencement of suitable treatment without delay.[5] Table 1.1 highlights the current diagnostic tests available in South Africa.

Table 1.1: Diagnostic tests available for TB in South Africa[5]

TEST	TYPE AVAILABLE	STRENGTHS	WEAKNESSES
MICROSCOPY	LED/Fluorescent microscopy	• High specificity • Short TAT	• Low sensitivity in people with low bacillary load (i.e. children and PLWHA)
CULTURE	Liquid Solid	• High sensitivity	• Long TAT • High contamination rates (liquid culture)
PCR-BASED ASSAYS	Line Probe Assay	• Short TAT • Detects RIF and INH resistance • High sensitivity for MDR-TB	• Reduced sensitivity in smear negative
	Gene Xpert or MTB/RIF	• Short TAT • Detects RIF resistance • High sensitivity for RIF resistance	• Does not detect INH resistance • Reduced sensitivity in smear negative

LED = light-emitting diode; TAT = turnaround time; PLWHA = people living with HIV/AIDS; PCR = polymerase chain reaction; INH = isoniazid; MDR-TB = multi-drug resistant TB; MTB = mycobacterium tuberculosis; RIF = rifampicin

1.2.6.2 Microscopy

Sputum-smear microscopy was developed more than a century ago and currently remains the most common method of diagnosing TB worldwide.[18] This method entails the microscopic observation of bacteria in sputum samples provided by the suspected TB-infected individual. The bacteria, otherwise referred to as acid fast bacilli (AFB), are identified by one of two staining methods, namely the Ziel-Neelsen (ZN) or fluorescent auramine staining. Approximately 80% of the results should be available from the laboratory within 48 hours, hence the short turnaround time (TAT).[5] However, due to smear microscopy often failing to detect active TB in individuals with either non-cavitary pulmonary disease or a low sputum-bacillary load (often immune-compromised patients), it is anticipated that molecular testing will soon replace microscopy as the first choice in TB diagnostics.[5] Table 1.2 describes the interpretation of various smear microscopy results.

Table 1.2: Interpretation of smear microscopy results[4,5]

ZN Staining		Auramine Staining	
Number of bacilli seen on smear	**Results reported**	**Number of bacilli seen on smear**	**Results reported**
No AFB per 100 oil immersion field	0	No AFB on slide	0
1 - 9 AFB per 100 oil immersion field	Scanty	<1 AFB per field	+
10 - 99 AFB per 100 oil immersion field	+	1 - 9 AFB per field	++
1 - 10 AFB per 1 oil immersion field (min 50 fields)	++	10 - 99 AFB per field	+++
>10 AFB per 1 oil immersion field (min 20 fields)	+++	>100 AFB per field	++++

ZN = Ziel-Neelsen; AFB = acid fast bacilli

An individual with a +++ ZN stain result will, therefore, be classified as more infectious than an individual with a + result. Sputum smear-negative individuals will, therefore, be less infectious than individuals showing a positive test of any kind.

The patients presenting with a smear-negative result are often difficult to classify clinically because there is a vast differential diagnosis. In addition, other chronic respiratory conditions may be misdiagnosed on the basis of a smear-negative sputum result.[46]

1.2.6.3 Culture

This method of TB diagnostics is considered to have a greater sensitivity than microscopy because it is able to detect a larger proportion of TB cases among symptomatic individuals.[5] Despite its accreditation as the apparent gold standard in TB diagnostics,[47] it does, however, present with a number of drawbacks that include high cost, slow turnaround time (TAT) and lack of accessibility to all. It is found to be useful in patients with paucibacillary TB (containing few bacilli), such as HIV-positive persons and children.[5]

1.2.6.4 Molecular testing / PCR-Based assays

This includes the molecular testing of a sputum sample and there are currently two different techniques used in SA, namely the Gene Xpert (GXP) and the Line Probe assay. Molecular testing is considered to be the future of TB diagnostics and is often regarded as the first choice in identifying a new suspect because it is able to diagnose both TB and drug-resistant TB.[5]

1.2.6.4 (a) Gene Xpert (GXP)

This test is commonly referred to as the Xpert MTB/RIF (*M Tuberculosis*/Rifampicin) and allows for the rapid identification of *M tuberculosis* in a sputum sample. This test has also recently been recommended by the WHO for its rapid results.[18] In addition, it identifies rifampicin susceptibility and resistance, which allows health care professionals to treat both uncomplicated and drug-resistant cases of TB aptly. The test is also specific for MTB and is, therefore, able to distinguish *M tuberculosis* from other mycobacteria.[5] Unfortunately, Xpert is unable to distinguish between live and dead bacteria and is thus limited to diagnostic status at present. Since Xpert has a higher sensitivity than smear microscopy, it is possible for an individual with TB to present with an Xpert positive test but produce smear-negative sputum.

Gene Xpert was rolled-out in South Africa in 2011, largely as a replacement for sputum microscopy given its sensitivity.[48] According to statistics released by the WHO in 2014, South Africa had 207 reported sites performing this molecular testing as of 2013, cementing its use as a primary diagnostic tool in the country.[5]

1.2.6.4 (b) Line probe assays (LPA)

This test is specific because it is used primarily on smear-positive sputum samples and isolates from both solid and liquid culture.[5] It is similar to the Xpert since it also detects MTB and rifampicin

resistance, but is additionally able to identify isoniazid resistance, which allows for the rapid diagnosis of MDR-TB. It is, however, labour intensive, costly and is dependent on smear results.[5]

1.2.6.5 Chest x-rays (CXR)

Chest X-rays are often an invaluable tool in patients who are unable to produce a satisfactory sputum sample, in HIV-positive individuals who tested negative via Xpert and in cases of EPTB (TB occurring outside of the lungs).[5] X-rays have previously been viewed as responsible for either over- or under-diagnosing TB and should, therefore, be interpreted together with a full clinical examination and reliable patient history.[5]

1.2.6.6 Other diagnostic tools available for use

These include, but are not limited to interferon gamma release assays (IGRA), blood culture, TB-LAM (lateral flow version), histological examination, tuberculin skin test, ultrasound, computerized tomography (CT scan), magnetic resonance imaging (MRI), erythrocyte sedimentation rate (ESR) and the test for adenosine deaminase (ADA).[5] These are, however, not as widely used.

1.3 RELATIONSHIP BETWEEN TUBERCULOSIS AND NUTRITION

1.3.1 Nutritional Response to TB

Any infection targeting a 'host', very often the human body, will result in a fierce battle between the host defences and the onslaught of the attacking organisms.[49,50] The severity of the metabolic response and concomitant tissue loss, is dependent upon the result of the conflict. The preliminary protection against attacking bacteria is mounted by the innate immune system, which is crucial in preventing infection dissemination to the remainder of the body.[51] Soon thereafter, the adaptive immune response takes over and generates detailed pathogen responses to combat the infection.[51]

As a result of infection with *M tuberculosis,* the body attempts to ward off the threat via the activation of cytokines, which are protein-like structures.[50] These cytokines can either be pro-inflammatory (TNF-α, IL-1β, IL-6, IFN-γ) or anti-inflammatory (IL-10, TGF-β, IL-4) in nature. The cytokines consequently activate a catabolic response in the body, often referred to as an 'acute phase response (APR)', which leads to a derangement in protein metabolism, accompanied by loss of both lean body mass and fat tissue. These metabolic manifestations can often be observed during other

cases of infection, such as HIV/AIDS or cancer, in which occurrences of cachexia are frequently described.

In a recent study conducted in South Africa, a group of newly diagnosed PTB patients was compared with a control group of patients presenting with various surgical/medical complications (similar stress response; although unrelated to TB).[52] It was noted that even though the control group was not 'healthy' in the conventional sense, the TB group still presented with higher levels of platelets, C-reactive protein (CRP), ferritin, cortisol and vital signs, indicating the propensity for a heightened APR in TB patients.[52]

1.3.2 Malnutrition and TB

Malnutrition is indicative of both over- and undernutrition, although the latter appears to be more synonymous with TB.[50,53] The complex relationship between the two conditions can be likened to a 'vicious cycle' because undernutrition can be hypothesised to be both a risk factor for TB disease, as well as a common consequence thereof.[54] Numerous studies have investigated the phenomenon of wasting associated with TB and its effects on body composition.[55,56] Tuberculosis was initially referred to as 'phthisis' (wasting away) by the Greeks and later termed 'consumption' in the 18th and 19th centuries by those attempting to illustrate the clinical changes in an infected individual.[50] Figure 1.1 highlights some of the main factors involved in the so-called 'vicious cycle' between TB and malnutrition.

Tuberculosis-induced wasting seems to be mainly due to loss of both fat mass and lean body mass.[57] Prior to the implementation of anti-tuberculosis chemotherapy, diet and enhanced environmental conditions were used as both defence and treatment against tuberculosis, thus highlighting the recognised importance thereof.[45,58]

Malnutrition can have a significant impact on the cell-mediated immunity (CMI) of the body, which is ultimately responsible for protection against TB.[53] Malnutrition, specifically protein energy malnutrition (PEM), has been highlighted as playing a contributory role in the progression of TB in terms of affecting the severity of the disease, reducing immune function, decreasing anti-tuberculosis medication efficacy, thus heightening mortality risk.[59] In an individual who has already been infected with TB but has not progressed from the latent stage, undernutrition may be the catalyst in the development of active TB.[60]

Some studies have shown an increased risk for developing active TB in underweight persons.[61,62] Recruits of the United States Navy showed a four-fold higher risk in those who were ≥ 10%

underweight at baseline, compared with their overweight counterparts.[61] Similarly, a large cohort study conducted with Norwegian participants older than 15 years showed a five-fold higher risk of developing TB among the lowest body mass index (BMI) group when compared with the individuals in the highest BMI group.[62]

1.3.2.1 Changes in anthropometrics / body composition

Some studies have shown that the presence of a low BMI and/or inadequate weight gain whilst on TB treatment increases the mortality risk in individuals,[60] and has an impact on treatment success rates.[63] A low BMI can also result in a heightened risk of developing various micronutrient deficiencies.[64] This TB-spurred weight loss can often be attributed to the following factors: reduced dietary intake due to periods of anorexia; increased energy expenditure;[50] nutrient losses from gastro-intestinal complications; medication side-effects; and metabolic modifications caused by the infection itself, as reflected in Figure 1.1.[60]

One of the proposed mechanisms for the metabolic changes seen during wasting in TB and other inflammation-driven states is known as the 'anabolic block'.[50,55] This describes the phenomenon often seen in tuberculosis patients, compared with healthy controls and non-infected malnourished persons, in which a greater proportion of protein (derived from oral intake) is used for oxidation and subsequently, the provision of energy compared with the more desirable endogenous synthesis of the important macronutrient.

Other studies have shown the high prevalence of impaired nutritional status of TB patients compared with healthy controls at baseline, often ranging from 20% to 71.6%.[59] Karyadi et al. showed that in 66% of patients presenting with PEM, the mean BMI of TB patients was 20% less than in healthy controls.[65] African data assessing the prevalence of a decreased BMI at baseline showed 71.6% of participants in Tanzania had a BMI <18.5 kg/m^2,[66] whilst a Ghanaian study demonstrated 51% of the participants to be below the same BMI cut-off point.[67] In addition, a Malawian study showed 57% of patients to be malnourished on admission.[68] It was, however, mentioned that the high rates of baseline malnutrition could be attributed to other confounding factors, and not necessarily solely to the TB disease.[67,69] These contributing factors could link strongly to the socio-economic climate of the population since developing African countries are often known to be strongly affected by issues such as extreme poverty and food insecurity with a spin-off effect of reduced health-seeking behaviours.[67,69]

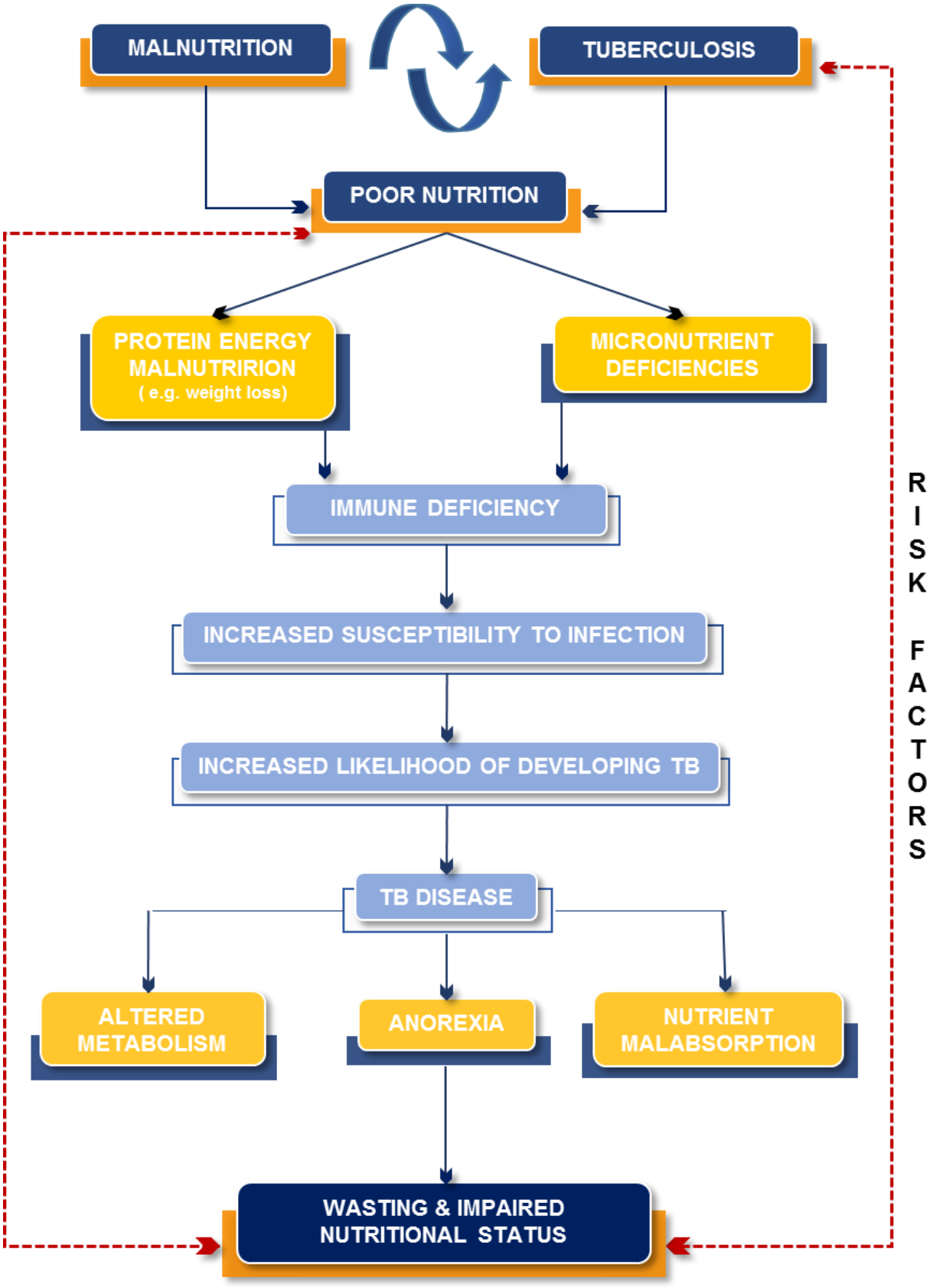


Figure 1.1: Flow diagram depicting 'vicious cycle' between malnutrition and tuberculosis

(Adapted from Kant et al.; Gupta et al.; Cegielski et al.)[45,53,70]

Epidemiological findings also seem to indicate that the presence of wasting or a reduced BMI on admission could result in increased mortality, as was shown in the previously mentioned Malawian study conducted by Zachariah et al., in which a BMI of less than 17.0 (moderate to severe thinness) was linked to a two-fold increased risk of early mortality.[68] Similarly, patients who had a decreased BMI at diagnosis, as well as those failing to gain more than 5% weight between time of diagnosis and end of treatment, were both significantly related to relapse risk.[61] Unfortunately, many patients who present with a decreased BMI upon admission often remain in this category even after completion of successful treatment.[66,71]

Findings have shown that individuals with TB disease often have lower levels of body weight, mid-upper arm circumference (MUAC), skinfold thickness, fat mass, lean body mass (LBM),[65] bone mineral mass,[55] arm muscle area (AMA), arm fat area (AFA)[56] and BMI,[56,72] when compared with healthy controls.[73] A study conducted by Villamor et al. in 2006 investigated the link between disease severity and anthropometry and found a noticeably worse prognosis for both daily activity scores and degree of disease in patients with an impaired nutritional status (low BMI, triceps skinfold and MUAC).[69]

In a study conducted by Paton et al., it was found that although wasting is considered synonymous with an overall loss of both LBM and fat mass, it appeared that the limbs (or periphery) suffered more LBM loss, whilst the trunk or central area showed more of a fat mass depletion.[55] Since the authors in this particular study investigated body composition in TB patients co-infected with HIV, they also proposed that TB, not HIV, seemed to be the main driving force behind the wasting phenomenon because the virus did not appear to have a noticeable effect on body composition.[55]

The literature also reports that several studies document weight gain in TB patients during the course of treatment,[54-56,71,73-76] but state that this is somewhat of a given arising from a successful treatment campaign. Whilst some studies have proposed that changes in body weight are linked to predictions of therapeutic success, especially in drug sensitive individuals,[77-80] others have alluded to the unreliability of using weight gain as a marker for successful treatment response.[66]

Despite reports of TB patients showing promising increases in weight whilst on treatment, there is some concern as to the composition of the weight gained. Although the most widely used form of assessing nutritional status remains either body weight or BMI, this often does not provide sufficient information as to the body composition of the patient.[81] This is where more specific measurements such as LBM and fat mass are invaluable.[82,83]

Another study by Paton et al. in 2004 showed that whilst the initial period of TB treatment produced a significant increase in LBM, the latter periods depicted the majority of weight gain to be due to fat mass, which is undesirable[57] because LBM gain is linked to beneficial aspects such as longevity, increased functionality and quality of life.[57,66,84] There could also be a gender-specific difference in body composition changes in patients suffering from TB.[81] It has been found that despite patients successfully completing their course of anti-tuberculosis therapy and showing an overall weight gain, this does not necessarily signify an increase in body protein, which can be deleterious for future health.[54]

Some studies have hypothesized that providing adjuvant treatment to TB patients in the form of nutritional intervention (macro- or micronutrient supplementation and/or nutrition education) may be important in the faster recovery from active disease, as well as an expedited improvement in nutritional status.[50,53,56-58,64,68,85] Whilst in theory, this reasoning may seem sound, a recent Cochrane review conducted in 2011 could not find sufficient evidence to support the provision of blanket nutritional interventions for TB-related PEM sufferers and recommended a greater number of large-scale studies be undertaken.[86] Additional recommendations include the possible benefit of targeting undernourished groups at risk of TB infection (children, the elderly, TB contacts, etc.) in the hope of decreasing incident TB cases,[58] although evidence is not conclusive in this regard. In seeming support of this recommendation, a local study performed at a facility in Cape Town, South Africa showed that provision of vitamin A and zinc in supplemental form resulted in no significant difference in the treatment outcomes of those with PTB at two months post-treatment.[87]

An initiative on behalf of the Nutrition Directorate of the Department of Health, suitably named the Nutritional Therapeutic Programme (NTP), aims to provide adults and children diagnosed with active TB and an impaired nutritional status with suitable supplementation in the form of an enriched porridge and/or oral drink. Qualifying criteria in the Western Cape Province for entry of adults into the programme include either an unintentional weight loss (>10% during the past six months or >5% during the past month) or a BMI<18.5 kg/m^2.[88] This programme (as is common with other supplemental feeding programmes) is however, not without its limitations in respect of both product utilisation by patients and distribution via the health facilities.

1.3.3 Micronutrient Depletion in TB

Appropriate assessment of micronutrient status is often lacking in TB studies. Micronutrients are postulated to play a role in impairment of immunity / weight gain, as well as interfere with treatment success and could therefore be considered a valuable component of a TB treatment plan.[57] Table 1.3 gives a brief summary of the important current micronutrient role players in TB disease.

Table 1.3: The role of micronutrients in immunity and tuberculosis

Micronutrient	Function	Findings in TB patients
Vitamin A	• T- and B-lymphocyte functioning • Macrophage activity • Involved in antibody responses and mucosal immunity[89,90]	• At risk of deficiency in TB [65,91-93] • Lower levels shown[64,94,95]
Vitamin C	• Anti-oxidant properties • Scavenging of ROS[96]	• Lower levels shown[95]

Vitamin D	• Modulates macrophage activity in the body[45]
	• Protects innate immune system against invasive or infectious pathogens[96,97]
	• Also used in early 20th century as part of TB treatment (diet or sunlight exposure) before aetiological aspects of condition were better understood[98]
	• Preliminary research = decrease inflammatory cytokines raised in IR[99]
		• Lower levels shown[100,101]
		• Systematic Review (2008) - Vitamin D levels 0.68 SD lower in individuals with TB than in healthy controls[101]
		• <u>Vitamin D deficiency:</u>
		  • Increase in peripheral IR
		  • Reduction in secretion of insulin from β-cells in pancreas and insulin sensitivity[99]
		  • Supplementation resulted in increased insulin sensitivity and secretion in some study populations[99,102]

Vitamin E	• Anti-oxidant characteristics • May have protective function against failure of T lymphocytes[96]	• Risk of deficiency in TB[103] • Lower levels shown[94,95]
Vitamin B6	• Synthesis of nucleic acid and proteins • Affects DNA and mRNA synthesis[96]	• Risk of deficiency in elderly TB patients[104]
Folate	• Synthesis of nucleic acid and proteins • Deficiency has profound effect on immunity[96]	• Risk of deficiency in TB[105,106]
Iron	• Involved in electron transfer, regulation of genes, oxygen reactions, cell differentiation and growth • Cytokine metabolism = production and action • Protein kinase C activation[96] • Deficiency could lead to anaemia (shown in many adults with PTB)[65]	• Lower levels shown[94,107] • Often linked to low haemoglobin levels[52] and anaemia[65,107]
Zinc	• Vital role in various immune processes[108] • Deficiency = increased susceptibility to infections[58] • Anti-oxidant properties • Role in Vitamin A metabolism[45]	• Risk of deficiency in TB[65] • Lower levels shown[64,94,109-111]
Selenium	• Anti-oxidant or anti-inflammatory properties[58] • Necessary for cell-mediated and humoral immunity[112] • Function in redox regulation • Protects against DNA-related damage • Aids membrane integrity[96]	• Lower levels shown[64,94,109]
Copper	• Expansion and preservation of immune system • Can affect neutrophil, monocyte, T-cell functioning[96]	• Higher levels shown[107,109] due to positive acute phase reactant status

TB = tuberculosis; ROS = reactive oxygen species; IR = insulin resistance; SD = standard deviation; DNA = deoxyribonucleic acid; mRNA = messenger ribonucleic acid

1.3.4 Biochemical Changes in TB

Several studies have also documented the noticeable alterations in biochemical parameters in patients with active TB disease. The biochemical markers occurring most frequently in the literature are discussed below.

1.3.4.1 Cholesterol

A reduced cholesterol level among TB patients is a common phenomenon and has often been reported in literature.[45,113,114] This is, however, a similar bi-directional phenomenon to that of malnutrition and TB because it is unclear whether hypocholesterolaemia predisposes to TB or vice versa.[113] It has been hypothesised that cholesterol (or lipids) may play a vital role in mycobacteria survival since cholesterol is vital for macrophage and lymphocyte action and if these are not functioning at full might, it may result in the initiation or progression of PTB.[113] Recent results from animal studies published in 2011, however, suggested that the mycobacteria do not rely solely on cholesterol as an energy or nutritional source during times of infection.[115]

It is also thought that prolonged persistence of mycobacteria may over time lead to cholesterol breakdown.[116] Similarly, patients with higher levels of cholesterol have been shown to have less severe radiological symptoms, with faster sterilisation of sputum after treatment commencement.[113,117]

1.3.4.2 Albumin

Serum albumin levels are often considered to be a reliable marker of nutritional status.[118] Additionally, during times of extreme stress, such as trauma, surgery, burn injuries or inflammatory states,[119] albumin is often considered a ‘negative’ acute phase protein, which indicates the tendency of the protein to decrease noticeably during a typical APR in the body.[52,111] Some studies have shown lowered albumin levels among TB patients when compared with healthy controls,[114] whilst a study conducted in Brazil between 2001 and 2003 credited depleted albumin levels at hospital admission to be independently linked to in-hospital mortality rates among TB patients.[120] Albumin levels have also been documented to increase with time whilst on anti-tuberculosis therapy.[107]

1.3.4.3 Pre-albumin

Although not as widely documented, pre-albumin is also a 'negative' plasma protein but presents with a shorter half-life than albumin and is generally considered to be a more sensitive marker.[121] Reduced levels of this compound have been reported in TB patients.[121]

1.3.4.4 C-reactive protein (CRP)

As is albumin, CRP is also an acute phase protein, although conversely, it is a 'positive protein', meaning that it increases in times of stress. It is also a non-specific measure of systemic inflammation in the body.[114] Raised CRP levels have been observed in several studies conducted among TB patients,[52,114,122-124] with reduced levels detected over time concomitant to treatment administration.[46,107,122-126] Although markers such as these are not able to diagnose certain conditions, they may be utilised when differentiating between inflammatory and non-inflammatory conditions or in the understanding of inflammation intensity,[46,119] which can be helpful in assessing treatment response in TB.[123]

1.3.4.5 Erythrocyte sedimentation rate (ESR)

Another marker of the acute phase response, ESR, which is associated with fibrinogen concentration,[119] performs similar to CRP in times of stress. However, the use of CRP levels when evaluating a patient is preferred because the ESR seems to have a slower response to a change in condition and appears to have a longer half-life when compared with CRP.[119]

1.3.4.6 Cytokines

These compounds are generally described as polypeptides that are involved in various signalling processes between activated body cells.[119] Cytokines play an invaluable role in the mediation of both the immune and the inflammatory response in the host, which is of crucial importance during mycobacteria infection.[123] Cytokines are very often a by-product of macrophages/monocytes, and some (mainly pro-inflammatory cytokines) play a key role in the generation of the acute phase proteins (i.e. CRP).[119] Raised cytokine levels are often linked to a reduced BMI, decreased appetite, and certain micronutrient deficiencies,[64,65] as well as to the severity of infection.[127] It is important to note that the types and amounts of cytokines, as well as the degree of the APR, differ according to the type of inflammatory disorder;[119] noticeable changes in cytokine levels have been observed in TB patients, which is indicative of the inflammatory state present. Increased levels of interleukin-6 (IL-6),[65,124,127-129] tumour necrosis factor-α (TNF-α),[123,127] interleukin-10 (IL-10)[128-130] and interleukin-

1 receptor antagonist (IL-1ra)[65] have been described. Although studies regarding TB have shown inconclusive results for certain cytokines such as interferon-gamma (IFN-γ), there also seems to be evidence of diminished cytokine levels with the extended duration of treatment.[123,124,131]

There has recently been investigation into the role of leptin and ghrelin in malnutrition seen in TB. Leptin is largely implicated as a protein hormone that exerts its effects by reducing the appetite and causing anorexia,[132] whilst ghrelin has the converse effect of improving the appetite.[133] These hormones have also been investigated concerning their role as regulators in the immune system.[133,134] Findings regarding the interaction of these compounds in TB have to date been conflicting, with some studies showing higher leptin levels in TB patients[135-137] and others showing lower levels.[40,64,138,139] Investigations into ghrelin levels in TB patients have also yielded conflicting results.[127,135,140,141] More research is, therefore, clearly needed in this area to understand further the process of cytokine-driven and hormone-assisted wasting in TB.

1.4 INSULIN RESISTANCE (IR)

1.4.1 What is Insulin?

Insulin is a peptide-based (or protein-based) hormone that has numerous functions, which include its primary function of glucose homeostasis in the body,[13,142] its function of stimulation of cell growth and differentiation and its roles in lipid and protein metabolism and storage.[13] A deficiency of insulin (as is often seen in type 1 diabetics) can have disastrous effects because it can lead to wastage of protein, ketoacidosis and ultimately, mortality.[143]

During a fasting state, no insulin is secreted, and glucose is generated for energy via the biochemical processes of gluconeogenesis and glycogenolysis.[144] Conversely, as soon as an individual has consumed exogenous dietary sources, insulin is released from the pancreas and thus inhibits the previous gluconeogenesis and glycogenolysis pathways, in order to maximise glucose uptake and distribution.[144]

1.4.2 Definition of Insulin Resistance

Insulin resistance is defined as “a condition in which the cells of the body become more resistant to the effects of insulin”.[8-11] It can furthermore be described as a “state in which higher than normal concentrations of insulin are required for a normal response”.[12] As a result of the ineffective action of insulin on both the peripheral and hepatic cells of the body, the glucose remains in the

bloodstream, which in turn results in the hyperinsulinaemic conditions mentioned above. Unfortunately, this is not without consequences because a high output of insulin often weakens the pancreatic β-cells, leading to constant hyperglycaemia and the progression to type 2 diabetes.[9] It has also been shown that individuals who are normoglycaemic but have high levels of plasma insulin are often already suffering from IR.[9]

A similar term, namely insulin sensitivity, is often referred to in literature. It can be defined as the efficacy of insulin to lower the blood glucose levels via promotion of glucose absorption into muscle and adipose tissue.[144] It also entails increasing glycogen storage in the liver and in so doing, decreasing glucose production in this organ.[10] The concept of insulin sensitivity differs from IR because it is the reciprocal form of IR.[6] Therefore if an individual is said to have 'normal insulin sensitivity', it would imply that the person has a normal insulin and glucose metabolism in their body. Similarly, if one suffers from IR, it is implied that they have a decreased level of insulin sensitivity, especially in the peripheral areas.[13]

1.4.3 Prevalence of Insulin Resistance

Several studies that determined the prevalence of IR in the general population found rates of the condition (and other associated morbidities) to be as high as 20 - 40% of persons, the majority of whom were classified as healthy.[145-146] Furthermore, an estimated 25% of the non-diabetic population in developed countries (i.e. not South Africa) is suspected to be as insulin resistant as their type 2 diabetic counterparts.[14]

In comparison, the International Diabetes Federation (IDF) estimates that approximately one-quarter (20 - 25%) of the global adult population is suffering from the metabolic syndrome (discussed at a later stage),[9] whereas in the United States (US), this estimated prevalence rises to an alarming 40%.[147] Other North American and European data has estimated the prevalence of the syndrome to be between 15% and 25%.[148] The occurrence of the metabolic syndrome also seems to be related to age, gender and ethnicity since prevalence rates differ between different populations and groups.[149] The syndrome tends to occur more among males and older patients (>50 - 60 years).[148]

1.4.4 Postulated Development of Insulin Resistance

Although IR is not classified as a disease or having a specific diagnosis, it has been implicated in the aetiology of morbidities such as cardiovascular disease (CVD), hypertension, polycystic ovarian syndrome (PCOS), type 2 diabetes, obesity and metabolic syndrome.[14,15,150,151]

The aetiology of IR is often attributed to being of either genetic or environmental causation.[15] Genetic factors could lead to a syndrome-related form of IR, whilst environmental aspects can result from dietary intake, low levels of physical activity, aging, smoking or specific drug use (including the use of thiazide diuretics, beta adrenergic antagonists and glucocorticoids).[15,152] Although the development of primary IR in individuals of normal body weight is possible, IR is mainly associated with obesity, especially with excess amounts of visceral adipose tissue (VAT).[12,15,153] The mere condition of being overweight (BMI >25 kg/m^2) has been shown to increase the risk of developing type 2 diabetes threefold.[154] Obesity is also very often associated with a chronic, albeit low level of inflammation in the body,[17,155] which in turn, has been shown to have a deleterious effect on insulin signalling pathways.[156]

Studies have shown that the presence of visceral obesity (intra-abdominal obesity or central adiposity) may be an even greater determinant of IR, diabetes and cardiovascular disease than a generalised obesity state measured by BMI.[157-162] Not only is VAT responsible for increasing free fatty acids (FFA) and triglycerides in peripheral muscle, but it is also linked to a reduced production of adiponectin (an anti-inflammatory cytokine), which is a molecule accredited with anti-diabetic, anti-atherosclerotic and anti-inflammatory properties,[163] all of which would prove useful in reducing the risk of IR development.

Insulin resistance typically ensues as a result of FFA release from visceral tissue, concomitant to the release of various hormones and cytokines from adipose tissue.[164] It is due to the latter that IR is often considered to be an inflammatory state since associations have been found between IR and increased levels of inflammatory markers such as TNF- α, IL-6, macrophage chemo-attractant protein-1 (MCP-1), CRP, resistin and plasminogen activator inhibitor-1 (PAI-1).[144,165-167] It can thus be postulated that because TB is considered to be an inflammatory state,[19] so too are IR and obesity.[155] The chronic, low-grade form of inflammation often seen in obesity has also been postulated to be the determinant of the type of micro-organisms found in the gut.[155]

Upon closer investigation of the effects of FFA relating to IR in the body, it can be seen that in addition to impeding glucose oxidation and transport, FFAs can also lead to atherogenic dyslipidaemia.[9] This process ultimately results in increased triglycerides and apolipoprotein B (ApoB) as well as reduced high density lipoprotein (HDL) cholesterol, all of which are synonymous with the metabolic syndrome.[9]

Other factors have also been shown to correlate positively with IR.[168] These factors include: anthropometric measures (BMI, waist and hip circumference); biochemical measures (raised fasting triglycerides, lowered HDL-cholesterol, glucose, insulin and hepatic enzymes);[169] clinical

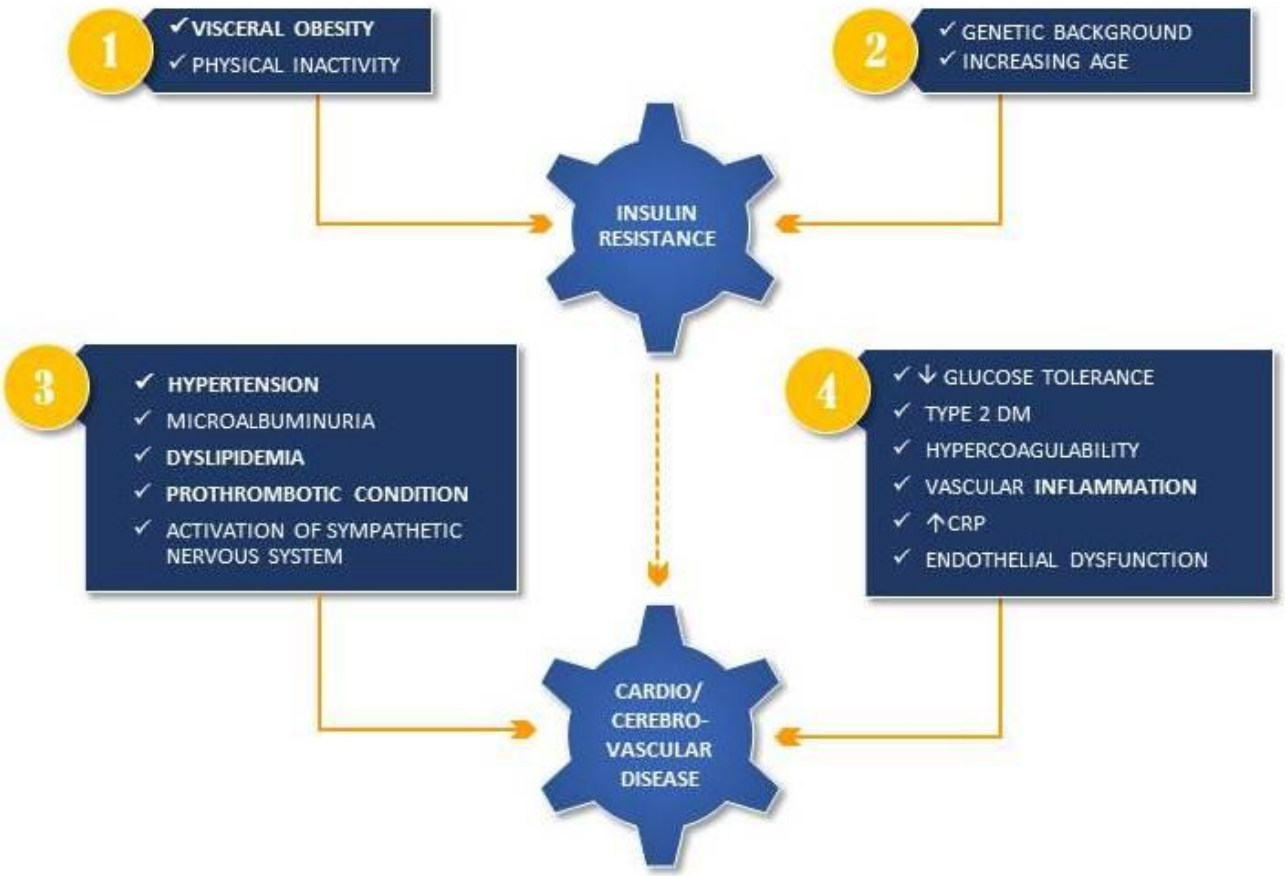
manifestations (acantosis nigricans, visceral obesity, acne, hirsuitism and hepatic steatosis)[15,149,170] and additional variables such as blood pressure and family history of diabetes.[171-174] These variables can be seen as being risk factors for the development of IR (e.g. increased BMI or abdominal obesity) or as a direct result of the IR state in the body, often seen in the clinical and biochemical presentation of the individual.

There are several factors involved in altering insulin sensitivity/resistance, such as recent physical activity or exogenous dietary intake. It is thus imperative that these are taken into account before any measurements evaluating IR status are commenced. Means of avoiding exercise and correct fasting procedures should be adequately communicated to study participants prior to testing.[13]

1.4.5 Metabolic Syndrome

The metabolic syndrome, also referred to as Syndrome X,[14] the Deadly Quartet[175] or the insulin resistance syndrome,[176-177] was first proposed in the late 1980s by Gerald Reaven in his Banting lecture.[14] It is characterised by the following indicators: abdominal obesity, atherogenic dyslipidaemia, elevated blood pressure, IR (with glucose intolerance), a pro-inflammatory state and a pro-thrombotic state. All are indicated in Figure 1.2.[14-17]

Insulin resistance is considered by many to be the epicentre of the metabolic syndrome storm because it is regarded as a key component in the development of the condition. Figure 1.2, adapted from Van Zwieten et al.,[148] depicts the various processes that are linked to the metabolic syndrome.



DM = diabetes mellitus; CRP = C-reactive protein

Figure 1.2: Pathological processes involved in the metabolic syndrome[148]

1.4.5.1 Diagnostic criteria of the metabolic syndrome

The 2006 IDF criteria (shown in Table 1.4) were largely born through the WHO, the National Cholesterol Education Program – Third Adult Treatment Panel (NCEP ATP III or ATP III criteria) and the European Group for the Study of Insulin Resistance (EGIR) due to the lack of standardisation for existing definitions. These guidelines are shown in Table 1.4.

It is clear that the core aspects of these are similar to the IDF guidelines (central obesity, dyslipidaemia and irregularities in glucose tolerance), although the WHO considers the presence of IR and microalbuminuria, as well as central obesity measured through waist:hip ratio. Interestingly, the WHO definition for IR states that it should be assessed under hyperinsulinaemic, euglycaemic conditions, and glucose uptake should be below the lowest quartile for the background population under investigation.[178] The American Association of Clinical Endocrinologists (AACE), also included

in Table 1.4, offers yet another definition of the metabolic syndrome, although this relies heavily on the subjective assessment of the particular clinician.[179] It has, however, been mentioned that the ATP III criteria are more user friendly than the WHO criteria, with the former being used more in daily clinical practice.[149]

Although the IDF guidelines are the most recent (2006), there is a lack of consensus as to which of the guidelines to utilise in diagnosing the syndrome, both on a global and national scale This is, in part, due to the lack of population-specific waist circumference cut-offs, especially for Sub-Saharan Africa. The IDF recommends persons in Sub-Saharan Africa be classified according to European cut-off points, which could prove problematic given noticeable differences in body composition.[180] The shortcomings of the IDF criteria have been noted by the consensus group, and mention is made that the definition will continue to change with the incorporation of new information.[180] Since the IDF and ATP III guidelines are very similar in their diagnostic criteria, these seem to be the most widely used (with the WHO definition in certain cases) and will be the tools discussed in this study.

In addition, the IDF has devised a secondary set of guidelines to be used in more complex research studies. These guidelines are shown in Addendum A of this study, and factors highlighted in bold indicate those tested for in this research.

Table 1.4: Available diagnostic criteria for the metabolic syndrome

Generalised metabolic syndrome Criteria	IDF Criteria[9,180]	ATP III Criteria[181]	WHO Criteria[178]	EGIR Criteria[182]	AACE Criteria[179]
Description of Criteria	*Increased waist circumference is a pre-requisite for IDF classification, combined with ≥ 2 of the other risk factors (increased triglycerides, decreased HDL-cholesterol, increased blood pressure and increased fasting glucose) or treatment for any of these conditions*	*≥ 3 of the generalised metabolic syndrome criteria mentioned need to be present for the ATP III classification.*	*In order to make a diagnosis of the metabolic syndrome, a patient must present with glucose intolerance, impaired glucose tolerance (IGT) or diabetes and/or IR, together with ≥ 2 of the following components*	*IR (defined as hyperinsulinaemia, top 25% of fasting insulin values among the non-diabetic population) plus two or more of the following*	*No defined number of risk factors is specified – diagnosis of the syndrome is thus left to the clinical judgement of the practitioner*
Waist Circumference	>94 cm (Male) >80 cm (Female)	>102 cm (Male) >88 cm (Female)		≥94 cm (Male) ≥80 cm (Female)	
Raised triglycerides	>1.7 mmol/L	>1.7 mmol/L	≥1.7 mmol/L	>2.0 mmol/L	≥1.69 mmol/L
Reduced HDL-cholesterol	<1.03 mmol/L (Male) <1.29 mmol/L (Female)	<1.03 mmol/L (Male) <1.29 mmol/L (Female)	<0.9 mmol/L (Male) <1.0 mmol/L (Female)	<1.0 mmol/L	<1.04 mmol/L (Male) <1.29 mmol/L (Female)
Increased blood pressure	>130/85 mmHg (and/or medication)	≥130/≥85 mmHg (and/or medication)	≥140/90 mmHg (and/or medication)	≥140/≥90 mmHg (and/or medication)	≥130/85 mmHg (and/or medication)
Raised fasting plasma glucose	>5.6 mmol/	≥6.1 mmol/L		≥6.1 mmol/L	Between 6.1 and 7.0 mmol/L
Impaired glucose regulation or diabetes			Present together with IR or singularly		2 hour post-glucose challenge: >7.7 mmol/L
IR			Under hyperinsulinaemic euglycaemic conditions – glucose uptake < lowest quartile for background population under investigation		
Central obesity			Waist:hip ratio >0.9 (Male) Waist:hip ratio >0.85 (Female)		
Overweight/obesity			BMI >30 kg/m^2		BMI ≥ 25kg/m^2
Microalbuminuria			Urinary albumin excretion rate ≥20 g/minute or albumin: creatinine ratio ≥30 mg/g		
Other factors	*European data for waist circumference used until such a time as more specific data for Sub-Saharan Africa becomes available*				Family history of type 2 DM, HPT or CVD PCOS Sedentary lifestyle Advancing age Ethnic groups having high risk for type 2 DM or CVD

IDF = International Diabetes Federation; ATP = Adult Treatment Panel; WHO = World Health Organization; EGIR = European Group for the study of insulin resistance; AACE = American Association of Clinical Endocrinologists; HDL – high density lipoprotein; IR = insulin resistance; BMI = body mass index; DM = diabetes mellitus; HPT = hypertension; PCOS = polycystic ovarian syndrome; CVD = cardiovascular disease

1.4.5.2 Complications and treatment of the metabolic syndrome

Persons diagnosed with the metabolic syndrome are often faced with a dire prognosis because statistics show that they have a two-fold greater chance of having a heart attack[148] and a three-fold greater chance of a stroke compared with healthy individuals. The more prevalent type 2 diabetes is also a significant threat because sufferers of the syndrome have a five times greater chance of developing the chronic condition.[9] This form of diabetes is synonymous with increased cardiovascular risk, as well as the premature development of various morbidities and ultimately, mortality risk.[183,184] In addition to the these morbidities, the metabolic syndrome can also result in the following life-altering conditions: cholesterol gallstones, sleep apnoea, polycystic ovarian syndrome (PCOS) in females, hypogonadism in males, microalbuminuria, left ventricular hypertrophy, fatty liver and certain types of cancer.[16,148] Unfortunately, the consequences of the metabolic syndrome (mainly due to diabetes and cardiovascular complications) have far-reaching implications on both the social and economic functioning of a country.

A multifaceted approach is needed in the treatment of the syndrome due to the multiple conditions found. The IDF recommends a rather aggressive approach in treatment in order to minimise the risk of developing cardiovascular complications and type 2 diabetes.[9,180] This strategy is two-fold since the primary approach entails the adoption of a healthy and sustainable lifestyle,[16,185] which encompasses a 5 – 10% intentional weight loss in the first year, as well as increased physical activity and appropriate dietary adaptations.[9,148,180,186] Nutritional management takes all aspects of the syndrome into account and focuses largely on weight reduction via a reduced energy intake, a low intake of saturated and trans fats, a high intake of dietary fibre, fruits and vegetables, a decreased intake of refined carbohydrates, and a reduced alcohol and sodium intake.[180,186] In addition, patients are strongly advised to stop smoking.[148]

The secondary and more radical approach consists of pharmaceutical therapy to treat the underlying components of the condition, namely antithrombotic medication, antihypertensives, oral antidiabetic agents (i.e. metformin), lipase inhibitors (orlistat) and lipid lowering medication (e.g. statins).[148,180] In extreme situations, behavioural therapy or bariatric surgery may also be implemented in the treatment of obesity.[148]

1.5 METHODS OF MEASURING INSULIN RESISTANCE

1.5.1 General

As can be seen by the multi-faceted effects of IR on varying disease aetiologies, it is imperative that reliable methods of diagnosing IR are identified. The following section of this literature review provides information on the current methods used as popular diagnostic tools for diagnosing IR as well as their respective techniques, strengths and limitations.

A variety of methods are currently available for the identification of IR status in individuals.[168,171] These include the gold standard method of the hyperinsulinaemic-euglycaemic clamp (HEC),[6,13,187,188] the frequently sampled intravenous glucose tolerance test (FSIVGTT) and other simpler, surrogate indices such as the homeostasis model assessment of IR (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) derived from oral glucose tolerance test (OGTT) data.[13,171] Whilst some of the methods mentioned above investigate peripheral (or muscle) insulin sensitivity, such as the clamp and FSIVGTT methods, the fasting indices consider insulin sensitivity in the hepatic tissues.[189-190] Although these fasting indices focus mainly on hepatic IR, they can also be considered valid when comparing them with the clamp method because there is a proposed close relationship between peripheral and hepatic IR levels.[146]

A detailed comparison of the most relevant methods used in the measurement of IR is given in Tables 1.5 to 1.7. Table 1.5 indicates the invasive indices (requiring intravenous insulin/glucose infusions), Table 1.6 depicts the non-invasive indices (measurements that do not require the administration of either exogenous insulin or glucose via the intravenous route) and Table 1.7 shows the fasting indices (utilising fasting measurements).

Table 1.5: Technique and uses of invasive IR tests

Name of IR test	Techniques & Uses	Advantages	Disadvantages	Interpretation of results
Hyperinsulinaemic-euglycaemic clamp (HEC)	• Considered to be the 'gold-standard' reference method of assessing insulin sensitivity in humans[6,187,191] • Conducted under very controlled circumstances • Intravenous infusion of insulin and glucose is administered for up to three hours (steady-state conditions) • Entire body glucose disposal calculated ('M')[190] • Insulin infused to create hyperinsulinaemic state, whilst glucose is concomitantly infused to maintain euglycaemia[191]	• First line test to evaluate insulin sensitivity in vivo • Direct measure of insulin action under steady-state conditions • Displays known precision and reproducibility[188,191] • Able to assess insulin secretion[192] • Can be used in all population types, including patients with diabetes (amount and rate of insulin infused can be adjusted according to the population[13,193] • Can differentiate between hepatic and peripheral insulin resistance[193]	• Specialised equipment (intravenous catheters, calibrated pumps and online glucose-level determination) • Suitably trained staff • Time-consuming (up to three hours/test) • Costly • Impractical for use in large epidemiological studies, clinical investigations or for routine clinical purposes[6,13,142,188] • Slight level of patient discomfort[13,142] • Not considered suitable when estimating normal insulin and glucose action • Viewed as 'un-physiological' = post-prandial dynamic conditions not portrayed[10,188,194] • Difficulty in repeating studies • Higher than normal insulin levels post infusion • Clamp instability[6] • No fixed consensus on important parameters when using the clamp method (optimal procedure duration, infusion rate of insulin and normalisation of glucose infusion rate) • Comparison between different studies utilising HEC challenging[195]	• IR estimated using ratio of mean glucose infusion: mean insulin concentration over previous 20 - 30 minutes of clamp procedure[6]

Name of IR test	Techniques & Uses	Advantages	Disadvantages	Interpretation of results
Hyperglycaemic clamp	• Derivative of HEC • Measures both β-cell secretory function and IR • Glucose infused at a rate to facilitate hyperglycaemic response[6,13]		• Not in accordance with normal physiological response • Not used as widely as its predecessor, the HEC[196]	
Frequently sampled intravenous glucose tolerance test (FSIVGTT)	• Indirect method of measuring insulin sensitivity/resistance • Glucose and insulin values are recorded during the test • Values computed by a mathematical model that investigates the relationship between the glucose and insulin and their kinetics[197] • Mathematical model is referred to as the minimal model analysis (MINMOD) • Test also requires subjects to have intravenous infusions of glucose / glucose and insulin, as well as frequent blood sampling[10]	• Analysis of both insulin and glucose levels is done using the computer program MINMOD = increased accuracy levels[142] • Also determines both early and late phases of insulin secretion from a single test[13] • Highly reliable and reproducible • Many international studies attest to its validity • Often referred to as the 'silver' standard after the HEC[198,199] • More cost-effective and less labour intensive to execute than HEC[6,198] • Able to identify and distinguish between different components of glucose disposal[10] • Describes more of a dynamic relationship between insulin and glucose[142]	• This method also requires a rather intense testing procedure • Subjects need intravenous catheterisation as well as frequent blood draws (up to 25 - 30 samples over three hours) • Limited use in large epidemiological studies[6,10,13,142,193] • Test depends on the accuracy and availability of the appropriate computer software = greater financial implications[13.142] • Reliability of results also diminished in known insulin resistant populations such as type 2 diabetics[13]	• Specialised computer program needed to analyse results[6]
Insulin suppression test	• Direct measurement of insulin sensitivity/resistance[200] • Test includes administration of somatostatin (a hormone preventing the release of other hormones) after an overnight fast, which subsequently inhibits endogenous insulin production[200] • Pre-determined amounts of glucose and insulin then infused at a constant rate	• First of its kind to use steady-state insulin levels to allow for disposal of a glucose load • Less labour intensive than the clamp method[13,188]	• Difficult to implement in large epidemiological studies or routine clinical settings[13,188] • Additional risks to the study population that include risks of hypoglycaemia and glycosuria in vulnerable groups[13]	

Name of IR test	Techniques & Uses	Advantages	Disadvantages	Interpretation of results
Insulin suppression test	• Recorded at set intervals during the test			
Continuous infusion of glucose with model assessment (CIGMA)[201]	• Based on principle of assessing the response of both insulin and glucose post-intravenous glucose infusion[193,201] • Glucose administered over one hour • Three phlebotomy samples required		• Not very widely validated or utilised in research studies thus far[193,201]	• Consultation with computer-assisted normogram or table[6]
Insulin sensitivity tests and short insulin tolerance test[6]	• Both tests used to identify possible insulin resistance following an intravenous administration of insulin • Insulin sensitivity test: Very similar to the HEC, but does not require such intensive sampling procedures • Short insulin tolerance test: Observes the rate of glucose decrease post-intravenous administration of an insulin bolus	• Both tests ultimately less labour intensive = executed over a shorter duration of time[6]	• Both tests not as well reported as HEC or FSIGVTT methods[6]	• Insulin sensitivity test: Steady-state plasma glucose calculated using mean of glucose levels over previous 30 minutes[6] • Short insulin tolerance test: Insulin sensitivity approximated from slope of regression line of logarithm of glucose concentration vs. time[6]

IR = insulin resistance

Table 1.6: Technique and uses of non-invasive IR tests

Name of IR test	Techniques & Uses	Advantages	Disadvantages	Interpretation of results
Oral glucose tolerance test (OGTT)	• Often the standardised test used in diagnosing glucose intolerance and type 2 diabetes in clinical practice[13,202] • Pre-determined amount of glucose (75g) – dissolved in 250ml of water is administered orally after an overnight fast • Glucose and/or insulin values are collected at specific times according to the purpose of the test (either clinical diagnosis or estimation of insulin sensitivity/secretion)[145] • Intends to determine the rate of glucose removal from the subject's blood stream[142] • Gives indication of insulin sensitivity in peripheral (not hepatic) tissues[13]	• Much simpler to perform than the invasive techniques (HEC, FSIVGTT, etc.) since glucose administered orally and not intravenously • More physiological than invasive methods[10,13,142] • Cost-effective	• Not an ideal 'typical meal situation'[13] • Labour intensive (Two hours per test) • Provides information regarding glucose tolerance of a subject, not IR[142] • Reason for devising surrogate indices that incorporate OGTT results to determine IR[13] • OGTT gives indication of insulin sensitivity in peripheral (not hepatic) tissues[13]	Once the OGTT has been performed under the correct conditions, the interpretation thereof is as follows:[3] • *Impaired fasting glucose (IFG): <7.8 mmol/L* • *Impaired glucose tolerance (IGT): 7.8 – 11.0 mmol/L* • *Diabetes: >11.1 mmol/L* After two hours
Surrogate indices derived from the oral glucose tolerance test (OGTT)	Different variations available (more precise calculations/formula shown in Addendum B) Avignon index: • Considers fasting insulin and glucose values taken prior to the test, as well as two hours post-test • Incorporates estimates of glucose Volume of Distribution (VD) = 150mL/kg body weight], which was then compared with the Bergman model as mentioned previously in this review[204] Belfiore index: • Utilises the Area Under the Curve (AUC) of both insulin and glucose[205] Gutt index: • Simply an adaptation of the Cederholm index (as can be seen in Addendum B)	• Unlike the OGTT alone, these indices are able to provide information on glucose tolerance, insulin resistance/sensitivity and insulin secretion • Efficacy of surrogate indices in measuring insulin resistance among a Finnish non-diabetic population investigated by Lorenzo et al. = found to be valid indicators[211] • However, three indices (Matsuda, Avignon and SI_{is}OGTT) = better correlation with HEC[211] • A similar study investigating surrogate indices and relation to cardiovascular risk factors in overweight and obese post-menopausal women = found	• Labour intensive • Rely upon reproducibility of OGTT • Lack of standardised reference ranges = inter-study comparisons not always feasible[13] • Insulin sensitivity not single-handedly responsible for altered plasma glucose and insulin values. These alternations can also be attributed to other biological processes (e.g. rate of glucose absorption in the body)[213]	

Name of IR test	Techniques & Uses	Advantages	Disadvantages	Interpretation of results
Surrogate indices derived from the oral glucose tolerance test (OGTT)	Matsuda index: • Utilises a combination of fasting and mean OGTT values to allow use in subjects with varying ranges of glucose tolerance • Both peripheral and hepatic insulin sensitivity are investigated[145] Stumvoll index: • Makes use of plasma insulin and glucose levels, as well as the body mass index (BMI) of a subject • Positively correlated with the HEC thus far[206] Oral glucose insulin sensitivity (OGIS): • Formula not shown in Addendum B (the appropriate online reference is given) because it is a more complicated formula due to varying parameters that differ according to author's/researcher's discretion SI_{is}OGTT • Shown promising validation results in recent studies conducted among middle-aged, inactive males[207] and non-obese, non-diabetic healthy participants respectively[208] Liver insulin resistance index: • Relatively new index, derived by Vangipurapu et al. in 2011[209] • Good correlation with the HEC[209] • Considers insulin values recorded during an OGTT, as well as high density lipoprotein (HDL) cholesterol and anthropometry measurements (such as BMI and body fat percentage)[209] Insulinogenic index (IGI): • Often used to determine pancreatic β-cell function (derived from OGTT)[210]	variability in inter-index results[212] • Quite specific study populations in studies mentioned above = therefore indices should be tested/validated in many other population groups with a variety of glucose tolerance ranges and insulin sensitivity *before* adopting into clinical practice[13]		

Name of IR test	Techniques & Uses	Advantages	Disadvantages	Interpretation of results
Surrogate indices derived from the oral glucose tolerance test (OGTT)	• Has not undergone stringent validation • Often used during initial stages of OGTT execution (first half hour) to determine insulin response as a result of a direct glucose challenge[142] <u>Glucose insulin (GI) product:</u> • Uses the product of plasma glucose and insulin concentrations obtained during the OGTT • Found by some researchers to display a good correlation with insulin-mediated glucose disposal rate of the HEC method[145] • Formula = the higher the levels of plasma glucose and/or insulin, the greater the presence of IR • Conversely, the lower the GI product, the higher the level of insulin sensitivity[142]			

IR = insulin resistance

Table 1.7: Technique and uses of fasting indices for testing IR

Name of IR test	Techniques & Uses	Advantages	Disadvantages	Interpretation of results
Fasting insulin	• Measurement taken after overnight fast (usually between 8 to 12 hours of fasting)[10] • Fasting insulin measurement (if >75th percentile of population) = previously considered by European Group for the Study of Insulin Resistance (EGIR) to be relatively good indicator of IR[214]	• Considered a practical means of measuring IR[215] • Very simple to perform • Inexpensive (true for all fasting indices)[13] • Viewed as being able to identify IR in individuals prior to development of clinical morbidity[142] • Healthy subjects with normoglycaemia, but concomitant hyperinsulinaemia, shown to display higher levels of IR, compared with those with normal levels of insulin[142] • Not true for glucose-intolerant persons / diabetics = typically present with hyperglycaemia but lower than normal insulin levels[142]	• Use of test is limited • Considerable lack of standardisation regarding insulin assay procedure (various assays used in laboratories) = difficulty with inter-study comparisons[142] • Large number of false-positive results recorded[142] • Fasting indices generally considered to be more reliable for normoglycaemic individuals than type 2 diabetics due to variability of fasting insulin values[194] • Formulae make use of >1 reference value (i.e. insulin *and* glucose or insulin *and* triglycerides) = more reliable than those that use insulin in isolation[194]	• Currently no available standardised cut-off reference values to identify IR by using fasting insulin measurement[193] • Cut-offs proposed but none accepted yet
Homeostasis model assessment (HOMA)	• *(Please see Chapter 2.6.3.4 for the HOMA-IR formula in question)* • Also known as HOMA-IR • First described more than a quarter of a century ago by Matthews et al.[7] • Proven useful in large epidemiological studies • Shows positive correlations with HEC[13,187,196,216] • HOMA-IR, together with QUICKI: two of the most popular fasting indices to determine IR[7,20] • Utilises both the fasting glucose and insulin values to determine basal IR[6]	• Surrogate of IR phenotype (identifies individuals with IR without measuring action of insulin)[219,220] • Simpler test • Not labour intensive or time consuming for the participant • Easy to calculate • More cost-effective • Minimally invasive for the participant[10,13,142,189] • Largely able to predict steady-state glucose and insulin levels in a fasting state[142]	• Measurements are not yet accredited as a standard for determining IR[171] • Applicability of all fasting indices seems weaker in healthy populations, normal weight individuals and those with primarily peripheral IR[13,190,223] • Lack of standardised reference values • Less relevance in certain ethnic groups[13] • Efficacy is often restricted in certain endocrinological abnormalities such as	• Lack of standardised reference values

Name of IR test	Techniques & Uses	Advantages	Disadvantages	Interpretation of results
Homeostasis model assessment (HOMA)	• Product of fasting glucose and insulin computed to determine predicted β-cell response and IR risk[13] • Mean of three samples (taken at five minute intervals) recommended = pulsatile nature of insulin release[7] • Single sample of insulin found to produce similar results in large research studies[196] • Adaptation of original HOMA formula to allow for addition of certain anthropometrical measurements (e.g. BMI) has shown improvements in IR identification[13] **<u>Alternatives for HOMA/HOMA-IR:</u>** <u>HOMA2:</u> • New and improved version of the original HOMA calculation[196] • Available via Oxford University on the following online link: www.dtu.ox.ac.uk/homacalculator/index.php • Also takes into consideration any possible differences in hepatic and peripheral glucose resistance[217] <u>HOMA-β:</u> • Formula designed to complement original HOMA formula • Allows estimation of insulin secretion by β-cells of the pancreas[7] <u>HOMA-S</u> • Adjusted HOMA formula • Relates to insulin sensitivity of an individual • Neither it nor HOMA-β formula can state with certainty whether an individual displays IR or not[196] <u>Log HOMA</u> • Noted that this formula often produces more comparable results (in relation to reference methods for IR) because fasting insulin levels not often normally distributed[13,194,218]	• Useful tool in epidemiological research • Validated by Insulin Resistance and Atherosclerosis Study (IRAS),[221] conducted among 1 460 individuals • Able to identify any variances in insulin sensitivity levels between different population groups[13] • Useful in long-term studies in which patients are followed up[6] • 'Fasting' indices (including HOMA, QUICKI, etc.) generally produce more reliable results among normoglycaemic individuals than those already presenting with type 2 diabetes[11] • Role of fasting indices in estimating cardio metabolic risk also valid = IR shown positive link to intima-media thickness (IMT) of carotid artery in cardiovascular-based research[222] (Important given strong link between IR and progressive development of cardiovascular disease	insulinoma and primary hyperaldosteronism[224] • Needs further validation in insulin-dependent individuals[142] • Lack of reliability of any of the fasting indices when investigating certain groups (e.g. elderly or uncontrolled or type 1 diabetics)[20,225,226i] • All fasting indices: Quality of output dependent on reliability of input measurements (namely insulin and glucose)[196] • Lower reproducibility than QUICKI (few studies)[227,228]	

Name of IR test	Techniques & Uses	Advantages	Disadvantages	Interpretation of results
Quantitative insulin sensitivity check index (QUICKI)	• *(Please see Chapter 2.6.3.4 for the QUICKI formula in question).* • QUICKI = inverse logarithm of HOMA • First described by Katz et al. in 2000, approximately 15 years after HOMA[20] • Positive correlations found between QUICKI and HEC in a varied population comprising non-obese, obese and type 2 diabetic individuals[20] • Whilst some studies have shown improved performance of QUICKI in relation to HOMA when validated against the clamp method,[229] others have shown no difference between the two fasting indices in relation to the FSIVGTT method of identifying IR[230] **<u>Alternatives for QUICKI:</u>** <u>Revised QUICKI – (R-QUICKI)</u> • Simply the original QUICKI formula with plasma free fatty acids incorporated into the equation • Allows for improvement in IR identification among a leaner population[231]	• Several studies showed promising correlations between its use and the HEC in a variety of study populations[20,218,226,,229,232,233] • Produces fairly consistent results • Minimally invasive technique[142]	• Same limitations as for HOMA since they are mathematically related • Significant lack of information regarding stimulation of glucose/insulin systems in the body because peripheral insulin sensitivity is not evaluated[10] • Validity in type 1 diabetics and measurements taken directly post-exercise also queried[20,234] • Problems identified in standardised insulin assays among different laboratories[235]	• Lack of standardised reference values • Normal reference ranges for QUICKI need to be established per laboratory[142]
Lipid-based fasting formulae	<u>McAuley index:</u> • Makes use of two variables (fasting insulin [µIU/mL] and fasting triglyceride [mmol/L] levels)[168] • These variables proposed to be most useful in predicting possible insulin sensitivity <u>TyG index:</u> • Devised by Geurrero-Romero et al.[236] • Utilises fasting triglycerides and glucose <u>Antuna-Peunte et al.</u> • Responsible for devising new index for use among mainly healthy subjects	<u>TyG index:</u> • Shown good correlation with the HEC[236]		

Name of IR test	Techniques & Uses	Advantages	Disadvantages	Interpretation of results
Lipid-based fasting formulae	• Primarily focusing on fasting non-esterified fatty acids (NEFA) and atherogenic index[13] *These lipid-incorporating formulae should, therefore, still be tested in the hope of increased identification of insulin resistant individuals.*			
Glucose/insulin ratio	• Utilises fasting glucose (G) and insulin (I) values in the formula G/I to calculate a suitable ratio[172,237,238]	• Used in several studies • Easily calculated	• Remains a theoretically imperfect index of insulin sensitivity/resistance Not widely used[142]	
Fasting insulin resistance index (FIRI)	• Index proposed by Duncan et al. in 1995 • Uses following formula to aid in calculation of possible IR: *FIRI = (fasting glucose x fasting insulin) / 25*[239]			

IR = insulin resistance; HOMA-IR = homeostasis model assessment – insulin resistance; BMI = body mass index

1.5.2 Research on Surrogate Indices for Insulin Resistance

A recent meta-analysis published by Otten et al. in 2014 investigated the correlation between surrogate measures of insulin sensitivity and the gold reference standard, the HEC.[240] A total of 120 articles were included in this meta-analysis, which reviewed the majority of the commonly used IR surrogate indices. The fasting indices that showed the strongest correlation with the HEC included the revised QUICKI (r=0.68), the QUICKI (r=0.61), the log HOMA-IR (r=-0.60) and the computer-generated HOMA for insulin sensitivity (r=0.57). Indices devised from OGTT-based values that showed the most promising correlation with HEC included the Stumvoll metabolic clearance rate (r=0.70), oral glucose insulin sensitivity (r=0.70), Matsuda index (r=0.67), Stumvoll insulin sensitivity index (r=0.67) and the Gutt index (r=0.65).[240]

1.5.3 Other Markers Under Investigation

With global interest in the timely identification of insulin resistant individuals in order to prevent further development of chronic disease, attention is now focused on alternative means of identifying IR. Of these alternative substrates, classic inflammatory markers are gaining popularity as possible markers of IR.[142]

These alternative markers include, but are not limited to the following (of which a comprehensive analysis is beyond the scope of this literature review): insulin growth factor binding protein-1 (IGFBP-1), soluble CD 36 (sCD36), CRP, ferritin, adiponectin, TNF-α, resistin, C3 complement, glycosylated haemoglobin (HbA1c), protein kinase C in microangiopathy and sex hormone-binding globulin (SHBG) in hyperandrogenic syndrome.[142]

1.5.4 Choosing the Most Appropriate Method for Measuring Insulin Resistance

Groop et al. highlighted five criteria with which an IR measuring tool should comply: (1) to achieve high enough insulin levels to stimulate glucose metabolism and to identify minimal differences in sensitivity of glucose uptake in relation to insulin; (2) to differentiate between hepatic and peripheral insulin sensitivity; (3) to record steady-state conditions adequately; (4) to rely on physiologically based notions regarding the body's glucose system; (5) to achieve some level of hyperglycaemia that is not un-physiologic.[241] The ideal method of 'diagnosing' IR should also allow for inter-individual comparison with

minimal risk to the persons involved, be cost-effective and be simple to perform.[241] As is clear from the analysis of available methods, no one tool stands out from the rest.

It has been mentioned that the best measure for assessment of IR is dependent on glucose status,[168] and this is, therefore, something that should be considered in the planning stages of a research study.[194] Similarly, the decision of which IR diagnostic measure to use is, therefore, not a decision to be taken lightly because there are many factors to take into consideration when choosing a suitable means of assessing IR. These factors include the study population under investigation, the proposed research question, the type of IR in question and available resources.[13,193,196] In considering all the resource-based factors having an effect on IR method selection, it is valuable to assess the impact of the time available for study completion, the budgetary constraints, available materials and equipment as well as the target sample size.[13,194] It is also of the utmost importance that one evaluates previous studies to gain more insight into appropriate methods used and to avoid preventable complications.[194]

It can be advantageous to make use of more than one IR index in a study both to corroborate findings between indices and prevent incorrect conclusions from being drawn.[194]

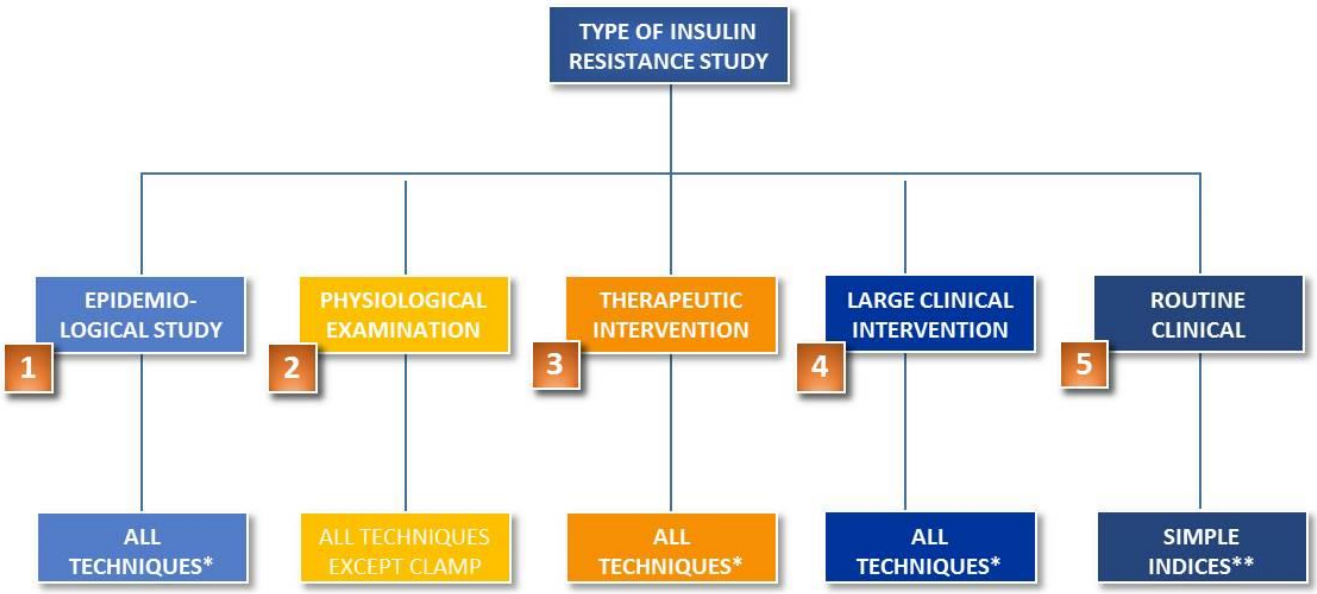
The following diagrammatical representations (Table 1.8 and Figure 1.3) depict certain considerations in the selection of an appropriate technique for the assessment of IR in research. Addendum C is another variation of selecting an appropriate tool.

Table 1.8: Criteria for studies in which non-invasive 'simple' indices of IR may be used[194]

Study objectives:
1. Large clinical practice and epidemiological investigations
2. Assessment of direct IR is not required
3. Outcome of IR is of secondary interest
4. Requirements of reference techniques unavailable (e.g. equipment, trained staff, budget)
5. Evaluation of new simple index
6. Investigation of validity and pitfalls of simple indices in specific clinical conditions

IR = insulin resistance

**Italicised and shaded criteria (viz. numbers 1 and 4 above) could be true for the current study.*



** All techniques include those mentioned in the review although the first choice remains the clamp technique, followed by the FSIVGTT and then the simple indices.*

*** Simple indices are used when the clamp technique and FSIVGTT are not possible to conduct.*

Figure 1.3: Types of insulin resistance studies[194]

It is important to note that if IR status is measured via the fasting indices mentioned above, additional means of assessing an individual's IR status should ideally be employed due to the stated limitations. Thus, the determination of classical risk factors such as those highlighted in both the IDF and ATP III definitions of the metabolic syndrome (waist circumference, blood pressure, lipid profile, etc.) should also be considered in conjunction with the results from fasting indices.[13,187]

1.6 RELATIONSHIP BETWEEN TUBERCULOSIS AND INSULIN RESISTANCE

If one considers the global epidemics of TB and diabetes, it seems that they have an inverse relationship. As the incidence of TB is slowly decreasing, the number of individuals suffering from diabetes is increasing at an alarming rate. This appears to be true in South Africa.[242]

The following section attempts to uncover more detail regarding the relationship between the two phenomena, which was first documented more than a thousand years ago.[243]

1.6.1 Tuberculosis Resulting in Insulin Resistance

It is hypothesised that the stress experienced during a significant long-term infection (such as TB or HIV) could result in increased IR. As a result of infection with the mycobacteria, macrophages, which form a vital part of the innate immune system, are often the first line of defence against these harmful organisms.[8] As is found with any infection, an increased amount of pro-inflammatory cytokines (such as IL-6 and TNF-α) is usually produced. This inflammatory attempt by the body to ward off possible pathogens such as TB might have a role to play in increasing IR, subsequently lowering insulin production and ultimately leading to hyperglycaemia in the host.[244] This process may also be accompanied by the release of certain stress hormones such as epinephrine, cortisol and glucagon, which further impair the action of insulin.[245]

In some cases, the hyperglycaemia resolves with duration of treatment,[246,247] but in other circumstances, may result in the development of a diabetic state or place patients at risk of developing DM at a later stage.[242] This condition is often referred to as 'transient hyperglycaemia', which occurs when glucose intolerance upon diagnosis subsides after active TB treatment.[248,249] The term 'stress hyperglycaemia' is also often used to describe the metabolic changes seen shortly after diagnosis of TB.[242] This may result in the erroneous classification of individuals as diabetic since the hyperglycaemia may, in fact, only occur due to the presence of the TB disease.[242,250,251]

Because HIV is also considered a chronic infection, much research has focused on the effects of the disease and the consequences of antiretroviral therapy (ART) on the body. Infected persons, especially those on ART, show a greater likelihood of developing the metabolic syndrome, which includes the component of IR.[22,252] In general, HIV infected patients have reported high rates of IR.[253]

There seem to be many cases investigating the prevalence of hyperglycaemia, impaired glucose tolerance (IGT) or DM in documented TB cases but as far as the researcher is aware, none appear to have specifically investigated IR prevalence in the same population.

A case-control study performed in 2011 in Tanzania showed an increase in hyperglycaemia among TB-infected cases, but not in the healthy controls.[254] It was postulated that these elevated levels could be a result of IR accompanying severe infection, as is found in TB.[255] Other studies have demonstrated a temporary increase in glucose levels in cases of active TB.[246,251] It has also been shown that infectious states can often lead to impaired glycaemic control in patients diagnosed with DM.[256]

In a local study conducted in the Eastern Cape Province of South Africa, differences between untreated TB patients (cases) and healthy, non-infected controls were assessed.[257] Results documented higher levels of glucose and insulin among cases compared with the controls. A delay in the clearance of insulin was also found, suggesting a defective internalisation of insulin.[257] These results seem to suggest that insulin-glucose metabolism is altered in patients with TB. This is in agreement with findings of an older study by Karachunskii et al. (1995), showing that carbohydrate metabolism is altered in TB patients, with emphasis placed on an enhanced secretion of insulin resulting in insulin deficiency and concomitant hyperglycaemia.[258]

In a case-control study published in 2014, researchers in Japan compared TB patients with non-diseased controls in order to assess the prevalence of glucose intolerance.[259] The study found a significantly higher prevalence of glucose intolerance among the TB cases compared with the controls (males: 34.2% vs. 14.4% in controls; females: 18.3% vs. 10.0% in controls).[259] Although the study found that those participants who displayed glucose intolerance had a higher BMI than their non-intolerant counterparts, the researchers did not exclude overweight/obese patients from their study population. Because HIV status was unknown at the time of recruitment, this remained unknown for the participants.

Rifampicin, one of the pharmaceutical agents used in the treatment of TB, has also been found to result in transient hyperglycaemia in the beginning stages of treatment due to it strengthening glucose absorption in the intestine.[260]

In a systematic review conducted by Jeon et al. in 2010,[251] a total of 30 studies were included in the review: 12 studies screened patients with diabetes mellitus for TB, and the remaining 18 studies attempted to screen TB patients for diabetes mellitus. The findings from this review showed that the diabetic patients had a TB prevalence rate of between 1.7% and 36%.[251] Those persons diagnosed with TB showed a diabetes prevalence rate of between 1.9% and 35%.[251] Both of these findings showed high rates of co-morbidities.

A research study conducted among the Indian population in the year 2000 provided researchers with the following statistics: Of prevalent PTB cases, 14.8% were classified as having diabetes, whilst an increased proportion (20.2%) of all smear-positive TB cases also suffered from diabetes.[261]

A spate of recent, globally conducted studies showed the heightened risk of TB patients displaying a higher prevalence of either DM or pre-DM. Viswanathan et al.[262] found prevalence rates of DM among

TB patients in India to be 25.3% (of which 9.3% of these were newly diagnosed diabetics) and pre-diabetic rates to be 24.5%. When compared with DM prevalence in the general population, it was much higher among TB patients.[262] Similarly, Balakrishnan et al. found 44% of their study population in India to have DM, which comprised 23% known diabetics and 21% newly diagnosed cases.[263] Upon perusal of DM prevalence in the United States and Mexico, Restrepo et al. found a 39% prevalence rate among TB patients in Texas, whilst Mexican TB patients displayed a slightly lower DM prevalence of 36%, although their study population included HIV-infected persons.[264] In the 2011 Tanzanian case-control study cited previously, TB cases had a DM prevalence rate of 16.7% versus the control group prevalence of only 9.4%.[254] When considering IGT rates in the same study, the TB group was again found to have a higher prevalence (37.6%) compared with 21.4% in the control group.

Another hypothesis that came to the fore many years ago involved the theorising of Schwartz, who hypothesised that the pancreas could be assaulted by TB by two possible means, namely concomitant pancreatitis (resulting in heightened susceptibility to inflammation and/or amyloidosis) or via the forced habitation of the pancreas.[265,266] It was, therefore, postulated that this could ultimately give rise to a greater incidence of diabetes among TB patients.

It is also suggested that patients with latent TB have a greater risk of developing active TB if they become IR during this time.[8] Moreover, the persistence of TB bacteria in adipose tissue has been hypothesised to be a possible causative factor for systemic IR.[267]

1.6.2 Insulin Resistance/Diabetes Resulting in Tuberculosis

The unforeseen association between DM and TB has long been suspected although possible explanations for its existence are not definitive as yet.[250,254,261,266,268-270] Before the discovery of insulin as a means of controlling blood glucose levels, life expectancy for diabetic patients was severely reduced, and the main cause of mortality was often found to be TB.[266] It has been suggested that an impaired immune system could be responsible for the development of TB[8] and sub-optimal blood glucose control,[152,271] but further investigation is still required. Persons with DM are often seen to present with a higher incidence of infections than non-DM patients as well as more infection-related complications,[272] of which one of the multiple causes is postulated to be defects in the innate immune system.[273] Diabetic patients often present with dysfunctional immune processes due to impaired macrophages, monocytes and T-lymphocytes, which can also affect the quality of phagocytosis.[274-276] This results in an impaired CMI, which is ultimately responsible for protection against TB.[274]

The effect of a poorly controlled diabetic state has been postulated to affect deleteriously the functioning of protective macrophages and lymphocytes, which can result in a diminished phagocytic attack against the invading mycobacteria.[250] Macrophages are normally tasked with the role of destroying any intracellular bacteria, but this is suggested to be altered in an IR state.[8,277] The macrophages develop a resistance to the higher insulin levels, leading to a decrease in phagocytosis activity. It is hypothesised that this can subsequently lead to increased survival of the mycobacteria within the macrophages, thus potentiating the risk for development of active TB.[8] Additional TB pre-disposing factors in DM patients include a deficiency of interferon gamma, pulmonary microangiopathy and a lack of various vitamins and/or minerals.[278]

Results obtained in a recent systematic review by Jeon et al. (2008) showed that persons with DM had approximately a three times greater risk of developing TB compared with those without DM.[269] In addition to this, it is thought that diabetes contributes to approximately 8% of incident tuberculosis cases each year.[38] Pulmonary tuberculosis is ranked ninth on the list of most frequent DM complications.[279]

Another systematic review conducted by the Jeon et al. group in 2011 showed that having active diabetes increased the risk of mortality, TB treatment failure and death combined, as well as the risk of TB relapse.[280] The systematic review highlighted the need for increased awareness of TB control programmes in diagnosing, treating and monitoring the dual burden of disease. This is largely being addressed via the Collaborative Framework for Care and Control of Tuberculosis and Diabetes,[38] which is affiliated to the WHO and the International Union Against Tuberculosis and Lung Disease.

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This is a worrying phenomenon because despite positive actions being taken to decrease the incidence of TB cases, the rapidly spreading diabetes epidemic may hamper these actions and result in sub-optimal TB care and monitoring.[242]

It is evident from the research that many of the findings are merely based on hypotheses at present, and that further, more conclusive evidence is essential in the understanding of these phenomena, namely, to ascertain which occurred first –TB or IR/DM.[250] This is often difficult to determine because many individuals are unsure of their DM/IGT status prior to diagnosis with TB.

1.7 CONCLUSION AND MOTIVATION FOR THE STUDY

The association between TB and the development of an IR state is somewhat of an enigma because evidence establishing or disputing the possible relationship is, to the knowledge of the researcher, currently not available. Since this study hopes to embark on novel research, especially in ambulatory PTB patients, it can be viewed similarly to a pilot project or front-runner in that it may give rise to similar research ventures and contribute to the available knowledge of the subject.

Given the nature of the TB epidemic in South Africa, and especially in the Western Cape, this study is definitely of relevance. South Africa is often viewed as having a double burden of disease and there is, therefore, great emphasis placed on the preventative care and treatment of the communicable disease aspect of this liability. This also correlates with the MDGs (due to be replaced with the SDGs in 2015), specifically Goal 6: Combat HIV/AIDS, malaria and other diseases.

If persons with TB were proved to exhibit a greater prevalence of IR compared with non-infected individuals, this would hopefully improve the current management of TB patients. Previously, much of the dietary treatment provided to persons with TB focused on curative care. New findings from this study could, however, signal a transition to a more preventative course of nutritional therapy induced action. Given the propensity of IR to result in CVD and DM, it would be beneficial to identify individuals at risk post haste.

The value of a multi-disciplinary approach in the treatment of IR in TB patients would thus be of critical importance because it would allow for medical, nutritional and other forms of therapy to aid in the ‘reversal’ or deceleration of the IR state, thus inhibiting the progression of TB disease and other IR-related conditions.

CHAPTER 2: METHODS

2.1 AIMS AND OBJECTIVES

2.1.1 Aim

The aim of this study was to determine if there is an association between tuberculosis and the development of insulin resistance in adults with pulmonary tuberculosis (PTB) in the Western Sub-district of the Cape Metropole region.

2.1.2 Objectives

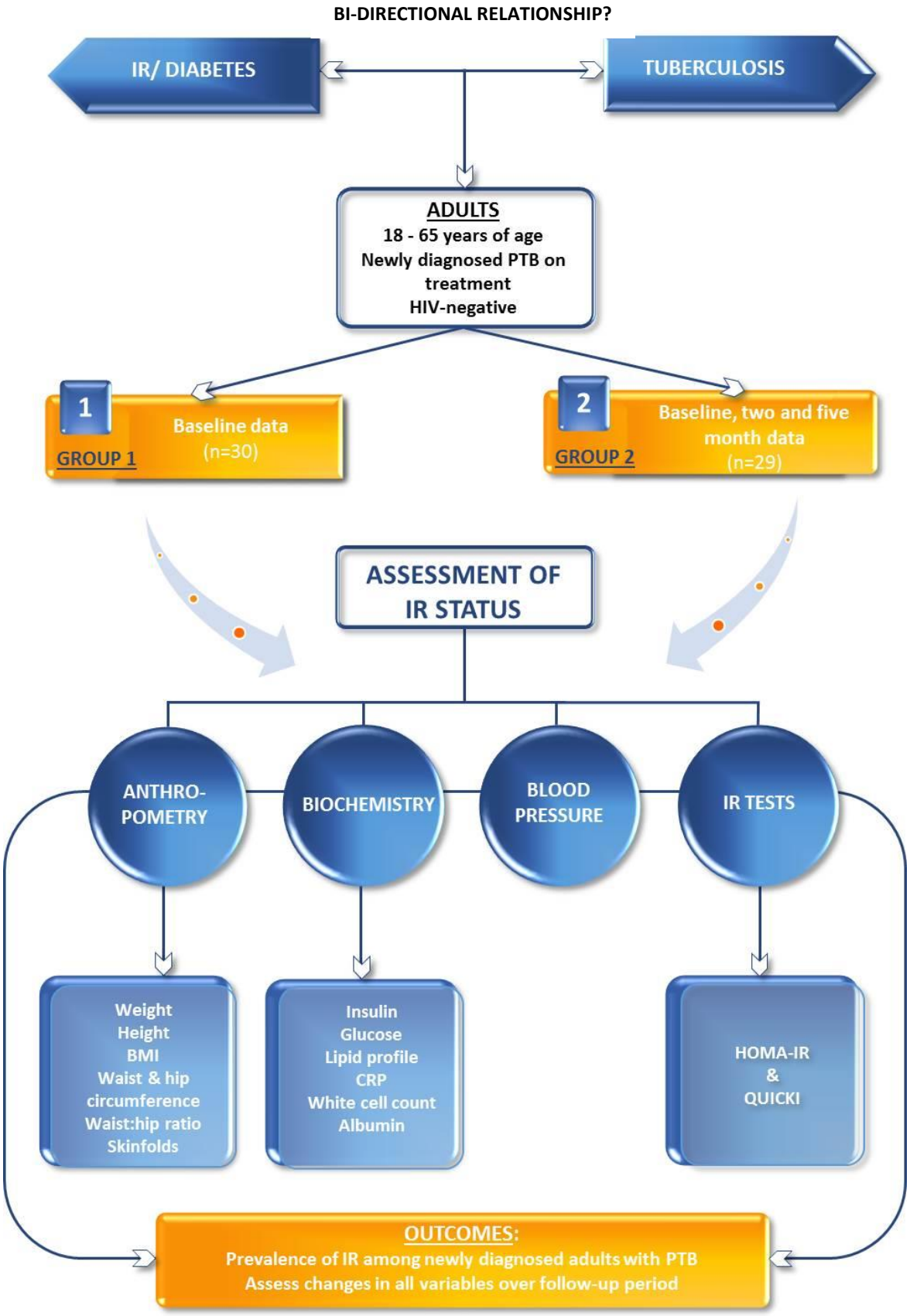
2.1.2.1 Primary objectives

- To assess if IR was present in participants with PTB through the testing of the following parameters:
    - Anthropometry
    - Biochemistry and blood pressure
    - Diagnostic IR tests
        - HOMA-IR
        - QUICKI

2.1.2.2 Secondary objectives

- To assess changes in anthropometrics and body composition at baseline and follow-up periods
- To assess changes in biochemistry and blood pressure at baseline and follow-up periods
- To assess changes in IR status at baseline and follow-up periods
- To assess changes in sputum TB results at baseline and follow-up periods
- To determine the relationship between IR status and sputum TB results at baseline and follow-up periods
- To assess differences between IR and non-IR participants
- To assess differences between sputum smear-negative and smear-positive participants

A conceptual framework of the current study is depicted in Figure 2.1 and highlights the study population, as well as the data collected from participants.



IR = insulin resistance; PTB = pulmonary tuberculosis; HIV = human immunodeficiency virus; BMI = body mass index; CRP = C-reactive protein; HOMA-IR = homeostasis model assessment–insulin resistance; QUICKI = quantitative insulin sensitivity check index

Figure 2.1: Conceptual framework of study

2.2 RESEARCH QUESTION

Are adults above the age of 18 years in the Western Sub-district of the Cape Metropole Region, with newly diagnosed PTB, at risk of developing insulin resistance?

2.3 HYPOTHESES

2.3.1 Primary Hypotheses

- There is no difference in anthropometrics or body composition in adults with PTB at baseline and follow-up periods
- There is no difference in biochemistry in adults with PTB at baseline and follow-up periods
- There is no difference in blood pressure in adults with PTB at baseline and follow-up periods
- There is no difference in IR status (via HOMA-IR/QUICKI) in adults with PTB at baseline and follow-up periods
- There is no difference in sputum TB results in adults with PTB at baseline and follow-up periods

2.3.2 Secondary Hypotheses

- There is no difference in anthropometrics, body composition, blood pressure or sputum results between IR and non-IR adults with PTB at baseline
- There is no difference in anthropometrics, body composition, blood pressure or IR status between smear-negative and smear-positive adults with PTB at baseline

2.4 STUDY PLAN

2.4.1 Study Type

A descriptive, cross-sectional study was undertaken. Due to the follow-up component of the study, a prospective cohort sub-section study was also performed. All data collected was quantitative in nature.

2.4.2 Study Population

The study population consisted of 59 ambulatory adult participants, who were enrolled in the study over a period of 17 months. Participant recruitment and concomitant data collection began in August 2013 and was completed in December 2014. The initial period of data collection was estimated to be approximately 10 months in duration. However, due to slower than expected recruitment rates, this was noticeably prolonged.

All eligible participants were recruited from the Albow Gardens clinic, by means of non-random, convenience sampling, during the collection period mentioned above. The clinic in question is under management of the City of Cape Town (CoCT), which fell under the local government of the Western Cape Province of South Africa. The initial proposed data collection site was not allowed access to by the Western Cape Department of Health (DoH) due to structural issues, which led to the researcher using the Albow Gardens facility. Use of the latter facility was subsequently approved by the CoCT and the Health Research Ethics Committee (HREC) of Stellenbosch University (SU). (See Chapter 2.8: Ethical and Legal Considerations).

Recruitment and screening of all participants was done on a weekly basis at the clinic by the principal researcher. Participants were identified and subsequently screened for compliance with the inclusion criteria for the study (See Chapter 2.5: Inclusion and Exclusion Criteria). Clinic staff also assisted with identification of new cases wherever possible. Participants were only deemed eligible for involvement in the study once all inclusion criteria were met and written informed consent was obtained (See Chapter 2.8: Ethical and legal considerations).

Data collection was performed a short distance away from the clinic, at the local TB hospital, Brooklyn Chest. This institution is under management of the provincial government of the Western Cape, namely the DoH. The majority of the data collection was completed by the principal researcher, and included administrative aspects (explanation and signing of informed consent, completion of data collection sheets (See Addendum D for data collection sheet), as well as performing the anthropometry measurements. A different venue to the recruitment site was arranged as to alleviate any burden placed on the clinic, as both space limitations and personnel shortages did not allow for effective data collection and participant comfort and confidentiality. The distance between the two facilities did not pose a problem, as it is standard practice for participants to move between the two sites (either by foot, private vehicle or public transport) if further investigations are needed. Once at the hospital, a separate facility

[TASK Applied Science TB research site– affiliated to Stellenbosch University (SU)] made their premises and trained personnel available to the researcher, as to not overload the hospital personnel.

Participants were selected for inclusion into either Group 1 (baseline measurements) or 2 (follow-up measurements) of the study. It was decided to include the first 30 participants that were recruited into Group 2, to reduce the duration of data collection. These selected participants were thus seen at baseline, two months and five months after treatment commencement. These follow-up dates were pre-selected during the planning stages of the study to coincide with the sputum follow-up dates at the clinic, in the hope of alleviating some of the loss to follow-up. Participants were also continually reminded of their return visits during the five month follow-up period, by means of telephone calls, mobile phone texts and home visits.

2.4.3 Sample Size

For statistical purposes, the total number of participants needed for the baseline aspect of the study (Group 1 and 2), was determined to be 60 participants. This was calculated using the estimation of a proportion with a 95% confidence interval and a precision/percentage error of 12.7%.

Furthermore, samples of size n=30 for the both Group 1 and 2 were intended to yield a power of 90% and detect an effect size of δ=0.64 [root mean square standardized effect (RMSSE)]. An effect size of δ=0.25 is regarded as small, δ=0.75 is regarded as medium and δ=1.25 is regarded as large. However, due to the loss of one of the original participants from the follow-up group in the final stages of data collection, Group 2 was reduced to 29 participants (See Chapter 3.1: Study population).

Sample size for Group 1 (n=30) therefore yielded a power of 90% and an effect size of δ=0.61 (RMSSE), whilst Group 2 (n=29) had an effect size of 0.62 (RMSSE). Therefore, a smaller than medium effect size could thus be determined with a sample size used in the study, which would allow the researcher to detect small to medium differences with a good confidence level.

2.5 INCLUSION AND EXCLUSION CRITERIA

Participants were included in the study if the following criteria were met:

- Males and females between the ages of 18 and 65 years
- Participants with newly diagnosed PTB, diagnosed via any of the following methods:
    - Molecular Testing [Gene Xpert (GXP) – PCR testing]
    - Microbiological (Sputum smear-positive swabs - AFB smears or cultures)
    - Radiography (Chest X-ray)
    - Clinical (productive cough >2-3 weeks; weight loss; fever; night sweats)
- Newly registered participants – commencing with a first course of TB treatment
- Participants placed on the standard baseline treatment protocol in accordance with CoCT / DoH guidelines
- Participants attending Albow Gardens clinic
- HIV-negative individuals
- Participants having given informed consent
- Participants willing to provide blood samples

Any potential participants who were found to have an unknown HIV-status were encouraged to undergo HIV counselling and testing (HCT). Re-evaluation for study participation was then investigated if a participant had a negative HIV-status after undergoing HCT.

Participants were excluded from the study due to the presence of any of the following:

- Individuals who were unable to speak/understand either English, Afrikaans or isiXhosa
- HIV-positive individuals (regardless of HIV treatment regime) or those of unknown status
- Individuals with any other form of TB (i.e. EPTB)
    - Abdominal
    - Military
    - TB meningitis
    - Pleural effusion
    - Multi drug resistant (MDR) or extensively drug resistant (XDR) TB
- Individuals with any pre-disposing IR conditions
    - Diabetes mellitus
        - Fasting blood glucose levels >7 mmol/L
        - Random blood glucose levels >11.1 mmol/L
        - Those with confirmed type 1 or 2 diabetes (with or without medication)

  - Polycystic ovarian syndrome (PCOS)
  - Metabolic syndrome
  - Pregnancy
- Individuals with severe underlying disease
  - Renal insufficiency
  - Cardiac insufficiency
  - Cancer, etc.
- Individuals with an increased BMI ≥30 kg/m^2 (i.e. obese individuals)*
- Individuals with an increased waist circumference*
  - Males >102 cm
  - Females >88 cm
- Individuals making use of any medications affecting IR status / glucose metabolism
  - Corticosteroids
  - Certain antipsychotic agents
- Any conditions affecting weight profile of patient
  - Pregnancy
  - Ascites
  - Oedema
  - Organomegaly
  - Tumours
  - Other forms of fluid retention
- Any conditions affecting body composition studies
  - Limitations for skinfolds
    - Participants directly post-exercise
    - Overheating

** Screening anthropometry was primarily obtained from the patient's folder (BMI), and additionally during face-to-face consultation with the patient.*

2.6 METHODS OF DATA COLLECTION

2.6.1 General Information

Data was gathered for every potential patient for each of the following:

- Age
- Gender
- Anonymous clinic folder number
- Diagnosis [according to the 10th revision of the International Statistics of Diseases and related Health Problems (ICD-10 codes)]
- HIV-status
- Treatment commencement date
- Sputum results (baseline, two and five months – if available)
- Contact details (mobile number, telephone number and/or home address)

Age and gender were obtained in order to describe patient demographics, whilst diagnosis, HIV-status and treatment commencement dates were gathered to ensure compliance with inclusion criteria. Patient privacy was ensured by the use of an anonymous folder number, but also allowed the researcher access to the linked records at the facility. Contact details were recorded for the follow-up participants, as to facilitate reminder telephone calls and/or home visits. All other recorded variables formed part of the data collection panel outlined by the researcher (See Chapter 2.6.3: Data Collected from Participants once Enrolled in Study).

2.6.2 Recruitment Process

As previously mentioned, recruitment of participants from Albow Gardens clinic was performed on a weekly basis by the principal researcher. Potential participants were identified according to new admissions in the facility's TB register, as well as faxed sputum results from the National Health Laboratory Service (NHLS). Participant's clinic folders were then screened for compliance with the inclusion criteria for the study.

The researcher made contact with the potential participants, either via a face-to-face encounter if the person was present at the clinic at the time of the researcher's recruitment visit, or otherwise, by means of a telephone call. Participants were subsequently given an appointment date and time to report to the

clinic and required to have undergone an overnight fast of ten hours to accommodate the biochemical testing. Participants recruited for the study were enrolled within a maximum time frame of two weeks after notification and/or treatment initiation, due to logistical issues and not desiring treatment to be a confounding factor.

2.6.3 Data Collected from Participants once Enrolled in Study

2.6.3.1 Anthropometry

The following anthropometrical data was collected and/or calculated for each of the study participants:

- Weight
- Height
- Body mass index (BMI)
- Waist circumference (WC)
- Hip circumference
- Waist:hip ratio (WHR)
- Elbow width (frame size)
- Body composition via skinfolds (triceps, biceps, subscapular, suprailiac)

2.6.3.1 (a) General

All anthropometrical measurements were performed by the principal researcher. There was, therefore, no need for standardisation of field workers. The researcher is a registered dietician (RD) and was adequately experienced to perform the above measurements.

The average of three measurements was taken for weight, height, waist and hip circumferences, as well as skinfold measurements. Height and elbow width were the only two variables to be measured at baseline, whilst all remaining anthropometrical measurements were performed at all follow-up visits. The BMI and waist:hip ratio were subsequently calculated once the necessary variables had been measured.

The calibrated beam-balance scale and fixed stadiometer were the property of the TASK Applied Science TB research site at Brooklyn Chest Hospital. The non-stretch tape-measure, elbow width calliper and skinfold calliper were the property of the principal researcher. Privacy of participants was

ensured by performing all measurements separately in an isolated room at the TASK site. The measurements were all carried out during normal working hours, in accordance with operating hours of the facility.

2.6.3.1 (b) Weight [2]

Body weight was recorded for each participant at each visit with the help of a calibrated, beam-balance scale. The participant was instructed to remove any unnecessary clothing and was weighed with the least amount of clothing possible. The participant was required to stand stationary and unassisted on the scale platform, with their weight firmly distributed on both feet. The measurement was read to the nearest 0.1kg.

2.6.3.1 (c) Height [2]

A fixed stadiometer was used to measure the height of each participant at the baseline visit. The participant was instructed to remove their shoes/socks and anything covering their heads before the measurement was taken. The participant was asked to place the heels of the feet together, with the legs straightened, arms to the side and relaxed shoulders. The head was positioned in the Frankfurt horizontal plane. The participant was requested to inhale before the measurement was taken to facilitate an accurate measurement. The headboard of the stadiometer was lowered onto the highest point of the head, with sufficient pressure to compress the hair. The measurement was recorded to the nearest 0.1cm.

2.6.3.1 (d) BMI [2]

This index was calculated for each participant by dividing the weight in kilograms (kg) by the height in metres squared (m^2). Each participant was classified according to Table 2.1 which depicts all the possible BMI classification categories. Participants in this study were however excluded if they had a BMI of ≥30 kg/m^2, thus ensuring that no participants fell into the obese categories at baseline.

Table 2.1: BMI classification – World Health Organization[281]

Interpretation	BMI Classification (kg/m^2)
Severe Thinness (Grade 3 Malnutrition)	<16.00
Moderate Thinness (Grade 2 Malnutrition)	16.00 – 16.99
Mild Thinness (Grade 1 Malnutrition)	17.00 – 18.49
Normal range	**18.50 - 24.99**
Overweight (Pre-obese)	25.00 – 29.99
Class I Obesity	30.00 – 34.99
Class II Obesity	35.00 – 39.99
Class III Obesity	≥ 40.00

BMI = body mass index

2.6.3.1 (e) Waist circumference [282]

A non-stretchable tape measure was used to determine the waist circumference of each participant. The participant was measured with no clothing covering the abdomen. The participant was instructed to stand upright, with his/her arms at the sides, feet together and abdominal muscles relaxed. The measurement was taken horizontally around the abdomen halfway between the last rib and the ileac crest. The measurement was taken after maximum exhalation, and the tape-measure fitted tightly around the abdomen, without compressing the skin. The measurement was recorded to the nearest 0.1cm.

Participants were classified according to the cut-off points shown in Table 2.2 below.

Table 2.2: Waist circumference classification - World Health Organization[282]

Waist circumference classification	Female	Male
Increased	>80 cm	>94 cm
Substantially increased	>88 cm	>102 cm

2.6.3.1 (f) Hip circumference [282]

The same non-stretchable tape measure used for the waist circumference was utilised for the hip circumference measurement. The participant was requested to remove any excess clothing around the hip area, and stand upright with his/her arms at the side and feet together. The measurement was taken in a horizontal position, at the widest part of the greater trochanters. The tape measure fit tightly around the hips, but did not compress the skin. The measurement was recorded to the nearest 0.1 cm.

2.6.3.1 (g) Waist:hip ratio [282]

This ratio was calculated by dividing the waist circumference (cm) by the hip circumference (cm).[282] Study-specific cut-off points for this variable are shown below in Table 2.3.

Table 2.3: Waist:hip ratio classification - World Health Organization[282]

Interpretation	Female	Male
Normal	<0.80:1	<0.90:1
At risk	≥0.85:1	≥0.90:1

2.6.3.1 (h) Frame size [2]

This variable was determined by the use of an elbow width measurement. The participant was requested to stand upright, with his/her right arm held up perpendicularly to the body. The forearm was flexed to allow the elbow to form a 90° angle. The participant's fingers were turned upward, with the palm facing the participant. A specialised elbow width calliper was used to measure between the lateral and medical epicondyles of the humerus. The soft tissue was compressed by the calliper and the measurement was read to the nearest 1 mm.

Frame size was then classified according to the elbow width and height of each participant by making use of Table 2.4.

Table 2.4: Elbow width classification for males and females of various heights[2]

Height (cm)	Small frame	Medium frame	Large frame
Males			
155 - 158	<64 mm	64 – 73 mm	>73 mm
159 - 168	<67 mm	67 – 73 mm	>73 mm
169 - 178	<70 mm	70 – 76 mm	>76 mm
179 - 188	<70 mm	70 – 90 mm	>79 mm
≥ 189	<73 mm	73 – 83 mm	>83 mm
Females			
145 - 148	<57 mm	57 – 64 mm	>64 mm
149 - 158	<57 mm	57 – 64 mm	>64 mm
159 - 168	<60 mm	60 – 67 mm	>67 mm
169 - 178	<60 mm	60 – 67 mm	>67 mm
≥179	<64 mm	64 – 70 mm	>70 mm

2.6.3.1 (i) Body composition via skinfolds

i) Skinfolds – General

All measurements were taken on the right hand side of the body. Each skinfold measurement (consisting of both skin and subcutaneous fat) was grasped approximately 1 cm above the mark indicating the skinfold site, and the skinfold was grasped between the thumb and index finger of the left hand. The skinfold calliper was calibrated to zero before each measurement was taken, and blades were placed in the middle of the base and top of the skinfold. The calliper dial faced upwards and the researcher was careful to read the measurement approximately two to three seconds after releasing the blades. The researcher also maintained pressure whilst holding the skinfold for the duration of the measurement. An interval of 15 seconds was allowed between measurements to allow the skinfold site to return to normal. Each reading was recorded to the nearest 1 mm.[2]

ii) Triceps skinfold

The researcher carefully calculated the middle point of the right arm, by measuring midway between the lateral projection of the acromion process of the scapular and the inferior margin of the olecranon process of the ulna. The elbow was bent at a 90° angle and the palm of the participant's hand faced upwards. The triceps skinfold measurement was taken on the posterior side of the right arm, over the triceps muscle. The measurement was taken at the same level as the midpoint of the arm, with the arm hanging loosely at the side. The skinfold was taken parallel to the vertical axis of the arm.[2]

iii) Biceps skinfold

The midpoint of the arm was also used in the measurement of this skinfold. The participant was seated for the biceps skinfold, with the right arm resting on the participant's leg and the palm facing upwards. The measurement was taken on the anterior side of the right arm, over the biceps muscle. The skinfold was taken at the same level as the midpoint of the arm and ran parallel to the vertical access of the arm.[2]

iv) Subscapular skinfold

The participant stood in an upright position, with his/her arms hanging loosely at the sides and feet together. The measurement was taken 1cm below the inferior angle of the scapular on the right hand side. The skinfold was taken at a 45° angle, and the skin was grasped 1 cm above and medial to the side along the axis.[2]

v) Suprailiac skinfold

The participant stood in an upright position, with his/her feet together and the left arm hanging loosely at their sides. The right arm was folded across the chest, to ensure that it did not impede the skinfold measurement. The measurement was taken 2 cm above the ileac crest, in the mid-axillary line, on the right hand side of the participant's body. The skinfold ran diagonally forward.[2]

vi) Analysis of skinfold measurements

Skinfolds were calculated using the average of each of the three measurements taken for the triceps, biceps, subscapular and suprailiac skinfolds. Certain skinfolds (triceps, subscapular and sum of triceps and subscapular) were interpreted according to standardised reference tables (percentile charts) and frame size, whereby appropriate classifications were made.[2]

vii) Body density

Body density was computed in order to indicate the percentage body fat of each patient. The sum of the four skinfolds mentioned above was entered into the age and gender appropriate formula depicted in Table 2.5 and the subsequent body density (D) calculated.[283]

Table 2.5: Equations for determining body density (D) according to age and gender[283]

Men:	Women:
17 - 19 years D = 1.1620 – 0.0630 X (log Σ4SKF*)	17 - 19 years D = 1.1549 – 0.0678 X (log Σ4SKF*)
20 - 29 years D = 1.1631 – 0.632 X (log Σ4SKF*)	20 - 29 years D = 1.1599 – 0.0717 X (log Σ4SKF*)
30 - 39 years D = 1.1422 – 0.0544 X (log Σ4SKF*)	30 - 39 years D = 1.1423 – 0.0632 X (log Σ4SKF*)
40 - 49 years D = 1.1620 – 0.0700 X (log Σ4SKF*)	40 - 49 years D = 1.1333 – 0.0612 X (log Σ4SKF*)
50+ years D = 1.1715 – 0.0779 X (log Σ4SKF*)	50+ years D = 1.1339 – 0.0645 X (log Σ4SKF*)

** Σ4SKF = Sum of 4 x skinfolds (triceps, biceps, subscapular, suprailiac) in mm*

viii) Body fat percentage

Upon calculation of the body density (D) value, the fat mass of each participant was calculated according to the following equation:[284]

$$\textit{Fat mass (kg)} = \textit{weight (kg)} \times [4.95/D - 4.5]$$

The percentage body fat was then calculated according to the following equation:[284]

$$\%\ \textit{Body fat} = [\textit{fat mass (kg)} / \textit{weight (kg)}] \times 100$$

Once this percentage value was obtained, it was interpreted according to the values shown in Table 2.6.

Table 2.6: Normal values for percentage body fat in adults[2]

Males:					
Age (years)	**Underweight**	**Slim**	**Satisfactory**	**Heavy**	**Excessive**
20-29	≤7	8-11	12-18	19-24	≥25
30-39	≤7	8-15	16-20	21-25	≥26
40-49	≤10	11-18	19-22	23-25	≥26
50-59	≤10	11-18	19-22	23-25	≥26
60-69	≤10	11-18	19-23	24-25	≥26
70+	≤10	11-18	19-21	22-25	≥26

Females:					
Age (years)	**Underweight**	**Slim**	**Satisfactory**	**Heavy**	**Excessive**
20-29	≤20	21-25	26-30	31-33	≥34
30-39	≤20	21-26	27-29	30-34	≥35
40-49	≤20	21-28	29-32	33-37	≥38
50-59	≤24	25-29	30-34	35-38	≥39
60-69	≤24	25-30	31-33	34-37	≥38
70+	≤21	22-28	29-33	34-36	≥37

2.6.3.2 Biochemistry

The following biochemical data was collected for each of the study participants at baseline and subsequent follow-up visits:

- Serum albumin
- White cell count (WCC)
- Fasting glucose
- Fasting insulin
- C-reactive protein (CRP)
- Lipid profile
    - Triglycerides
    - Total serum cholesterol
    - LDL–cholesterol
    - HDL–cholesterol

Table 2.7 depicts the necessary samples and equipment needed for the biochemical analysis of the above tests.

Table 2.7: Biochemical analysis performed by National Health Laboratory Services – samples and equipment[285]

Biochemical marker	Equipment used in analysis	Serum vs. plasma sample	Quantity of sample needed
Albumin	Siemens Advia 1800	Serum	1 yellow top tube (4 – 5 ml)
White cell count	Siemens Advia 2120	Plasma	1 purple top tube (4 – 5 ml)
Glucose (fasting)	Siemens Advia 1800	Plasma	1 grey top tube (1 ml)
CRP	Siemens Advia 1800	Serum	1 yellow top tube (4 – 5 ml)
Lipid profile	Siemens Advia 1800	Serum	1 yellow top tube (4 – 5 ml)
LDL-cholesterol	*Friedewald formula (using total cholesterol, triglycerides and HDL-cholesterol)*[286]	-	-
Insulin	Siemens Centaur XP	Serum (spun and frozen as soon as possible)	1 yellow top tube (50 microlitres)

CRP = C-reactive protein; LDL = low density lipoprotein

The above phlebotomy samples were taken by a professionally trained nursing sister employed by the TASK Applied Science TB research site. Blood samples were all collected on the morning of data collection after a ten-hour overnight fast. After the blood samples were drawn, the principal researcher transported them in suitable and secure containers, under the correct storage conditions (carefully ensuring samples were not haemolysed), to the laboratory at Tygerberg Academic Hospital (TAH). The laboratory is administered by the NHLS. Samples were duly analysed and results were conveyed via email to the researcher for capturing of data (See Addendum E for a copy of the form used for the NHLS). Blood volumes used for the biochemistry panel above were in line with normal quantities used in government health facilities across the country (did not exceed 15 ml per draw). There was, therefore, no additional ethical risk posed to participants enrolled in the study, as larger volumes of blood were not necessary, which is in line with guidelines in this regard.[285,287]

The methods utilised in the analysis of biochemical markers by the NHLS for this study are detailed in Table 2.8.

Table 2.8: Methods used in the analysis of biochemical markers[288]

Biochemical marker	Method/s
Insulin	• A serum sample to determine fasting insulin was used and the sensitivity/assay range fell between 0.1 – 300 mU/L • The assay used was the ADVIA Centaur® Insulin Lite Reagent and Solid Phase
Albumin	• This substrate was tested by means of using bromocresol green solution (BCG) as a binding dye and required a human serum and plasma sample
Cholesterol	• This method is based on an enzymatic method that makes use of cholesterol esterase and cholesterol oxidase conversion, followed by a Trinder endpoint • It also makes use of a human serum and plasma sample
Direct HDL-cholesterol	• This method was used to quantify the HDL-cholesterol value and consisted of two steps • (1) Separate all the 'non-HDL' particles from the sample • (2) Allow specific measurement of HDL-cholesterol • This was also measured in the serum and plasma
Triglycerides	• This method is based on the Fossati three-step enzymatic reaction, utilizing a Trinder endpoint • A human serum and plasma sample is also needed and was analysed by the ADVIA chemistry system

HDL = high density lipoprotein

The following cut-off points shown in Table 2.9 were used to classify biochemical values as normal, increased or decreased.

Table 2.9: Biochemical reference ranges – National Health Laboratory Services[289]

Biochemical value	***Normal range***	
Albumin	35 – 52 g/L	
White cell count	4.0 – 10 x 10^9/L	
Fasting plasma glucose	4.1 – 5.9 mmol/L	
Fasting insulin	3.0 – 25 mU/L	
CRP	0.0 – 10 mg/L	
Lipid profile		
Total cholesterol	≤5mmol/L	
Triglycerides	0.5 – 1.5 mmol/L	
Cardiovascular risk stratification and cholesterol targets		
	Category 1 risk*	**Category 2 risk****
Cholesterol	<4.5	<5.0
Triglycerides	<1.7	<1.7
HDL-cholesterol (males)	>1.0	>1.0
HDL-cholesterol (females)	>1.2	>1.2
LDL-cholesterol	<2.5	<3.0

CRP = C-reactive protein; HDL = high density lipoprotein; LDL = low density lipoprotein

***Category 1 Risk**[289]

- Established atherosclerosis
- Coronary heart disease
- Peripheral vascular disease
- Diabetes type 2
- Diabetes type 1 with micro-albuminuria or proteinuria
- Genetic dyslipidaemias e.g. Familial hypercholesterolaemia

****Category 2 Risk**[289]

- Primary prevention and all other categories of dyslipidaemia

In addition, the latest South African dyslipidaemia guidelines suggest the following for an initial ten-year CVD event risk (Table 2.10).

Table 2.10: LDL-cholesterol goals relating to 10-year cardiovascular disease event risk[290]

Cardiovascular disease event risk	LDL-cholesterol target
<15% ten-year CVD event risk	<3.0 mmol/L
15% - 30% ten-year CVD event risk	<2.5 mmol/L
>30% ten-year CVD event risk	<1.8 mmol/L

LDL = low density lipoprotein; CVD = cardiovascular disease

2.6.3.3 Blood pressure[291]

The blood pressure of each participant was taken by the same registered nurse responsible for the phlebotomy testing. This was done according to standardised methods as each participant was seated and relaxed, whilst the nursing sister placed an inflatable cuff around in the brachial artery of the arm. This cuff was then inflated until the pressure in the cuff surpassed that of the artery. The nurse then listened for the presence of Korotkoff sounds, with the first sound indicative of the systolic blood pressure and the subsequent disappearance of Korotkoff sounds signalled the diastolic blood pressure reading. Measurements were performed by the on-site sphygmomanometer belonging to the TASK Applied Science TB research site. All measurements were classified according to the values shown in Table 2.11.

Table 2.11: Classification of blood pressure in adults[292]

Classification	Blood Pressure (mm Hg)
Normal	<120/80
Pre-hypertension	120 - 139 / 80 - 89
Stage 1	140 - 159 (systolic) / 90 – 99 (diastolic)
Stage 2	≥160 (systolic) or ≥100 (diastolic)

2.6.3.4 HOMA-IR and QUICKI measurements

The HOMA-IR diagnostic test was performed according to the following formula:[7]

Fasting serum insulin (µU/ml) x fasting plasma glucose (mmol/L) / 22.5

The denominator (constant) of the above equation refers to the normalisation (based on normal fasting values). Laboratory values reported glucose values in mmol/L, whilst serum insulin values were reported in mU/L. The latter is not problematic as this equates to the same value when converting to µU/ml.[285]

The QUICKI measurement is simply the inverse logarithm of the HOMA-IR calculation, namely:[20]

1 / (log(fasting insulin µU/ml) + log(fasting glucose mg/dL))

In order to convert the glucose value from mmol/l to mg/dL, a conversion factor of 18.018 was used. (I.e. 1 mmol/L = 18.018 mg/dL or mg/dL x 0.0555 = mmol/L).[285]

The above formulae were entered into a Microsoft Excel spreadsheet and subsequent values were determined. As there is currently no standardised cut-off point for either the HOMA-IR or the QUICKI measurement, the data generated by this study was used to calculate a relevant HOMA-IR cut-off point. This was based on the lower limit of the upper quartile (P75), as has been performed in similar studies.[293-296]

Once the HOMA-IR cut-off had been calculated, the corresponding QUICKI value was determined by means of a receiver operating characteristic (ROC) curve analysis. Any individual having a HOMA-IR value greater than the calculated cut-off point, as well as below the QUICKI cut-off point, classified a

participant as having possible IR. The HOMA-IR was used as the primary tool for identifying IR and the QUICKI was calculated to reinforce or compare results.

2.6.3.5 Sputum results

The baseline sputum (smear result) of each participant was recorded as part of the eligibility screening performed by the researcher. Results were classified via ZN staining (as shown in Table 1.2). Participants that had a positive sputum result at baseline were required by DoH / CoCT guidelines to have repeat sputum tests at two and five months after treatment commencement. These results (if available) were also recorded by the researcher.

2.6.4 Reliability and Validity

2.6.4.1 Anthropometry

Reliability of anthropometric measurements was ensured by using a single, calibrated scale and a fixed stadiometer. The elbow width calliper, skinfold calliper and tape-measure used by the researcher for performing measurements were used throughout the duration of data collection. Reliability was further ensured through the use of the standardised measuring techniques explained above and taking the average of three measurements for the majority of variables. All of the anthropometrical measurements were performed by the principal researcher, therefore ensuring the absence of inter-observer variation.

Validity of body weight was ensured via adequate and regular calibration of the available scale. Formal calibration was performed by the TASK site on an annual basis and informal calibration was done by the researcher on a weekly basis by means of a known 1 kg and 10 kg weight.

2.6.4.2 Biochemistry

The analysis of biochemical samples was performed by the NHLS throughout the duration of the study. The NHLS is a South African National Accreditation System (SANAS) accredited laboratory and all the tests that were requested by the researcher appeared on the accreditation schedule of the NHLS. South African National Accreditation System accreditation stipulated strict proof of laboratory performance and documentation of all procedures in accordance with pre-set standards. Quality controls were run on an internal daily basis at six, eight or twelve hourly, depending on the sample under analysis. The laboratory

belongs to various external quality assessment (EQA) programmes (Thistle, BioRad, NHLS EQA and DEQAS).[285]

2.7 DATA ANALYSIS

2.7.1 General

Socio-demographic data was recorded on the specially designed data capturing form (Addendum C). Participant anthropometrical data and blood pressure values were also captured on this form. Results of the socio-demographic portion of the study (age and gender distributions) are graphically depicted in the results section (Chapter 3). Biochemical results were received electronically via email (researcher's work email address, to which only she had access).

Variables were recorded as continuous values for all measurements and basic descriptive statistics were used to identify trends and associations. This was done as differences among groups for certain variables (such as BMI), are more easily identified if the data is recorded in its continuous form, as opposed to categorical data.

Data was also transformed into categorical data at a later stage in order to assess for inter-group changes and to identify risk of IR development.

The relevant cut-off points for variables (anthropometrical, biochemical, blood pressure, HOMA-IR/ QUICKI etc. are depicted in Chapter 2.6.3).

2.7.2 Statistical Analysis

The researcher was assisted during the planning and statistical analysis stages by a statistician assigned by the Division of Human Nutrition of the University of Stellenbosch. Once data had been recorded on the data capturing sheet, it was captured onto a designed Microsoft Excel spreadsheet and duly submitted to the statistician for assistance with analysis. STATISTICA version 12 (StatSoft Inc. (2014) STATISTICA (data analysis software system, www.statsoft.com) was used to analyse the data.

Summary statistics were used to describe variables such as participant demographics. Distributions of variables were presented with frequency tables and/or histograms. Medians or means were used as measures of central location for ordinal and continuous responses and standard deviations and quartiles as indicators of spread.

The relationship between two continuous variables (such as weight and BMI) was analysed with regression analysis and the strength of the relationship measured with Pearson correlation or Spearman correlation (if continuous variables were not normally distributed).

The relationship between continuous response variables and nominal input variables (i.e. weight and gender), was analysed using appropriate analysis of variance (ANOVA) and appropriate repeated measures analysis of variance (RMANOVA) when responses were measured at specific time intervals. Variables were included as co-variates in appropriate analysis of covariance (ANACOVA) to account for possible confounding variables (e.g. age).

Non-parametric ANOVA methods were used when comparing ordinal response variables to a nominal input variable. The Mann-Whitney or Kruskal-Wallis tests were used for completely randomized designs. The relationship between nominal variables (such as gender and race) was investigated with contingency tables and appropriate chi-squared tests such as the Maximum-Likelihood (M-L) chi-square test or the McNemar test.

Bootstrap procedures (a computer performed re-sampling procedure performed on current data) were used in cases where the residuals were not normally distributed.[297] ROC curves were performed to calculate the corresponding QUICKI cut-off point at baseline. These analyses play a pivotal role in both the assessment and utilisation of various diagnostic tools, of which the HOMA-IR and QUICKI are two such examples.[298]

A p-value of $p<0.05$ represented statistical significance in hypothesis testing and 95% confidence intervals were used to describe the estimation of unknown parameters.

2.8 ETHICAL AND LEGAL CONSIDERATIONS

Initial ethical approval was granted by the HREC of SU for the study in October 2012 (Project number: S12/08/227). Due to problems experienced with gaining approval from the DoH for the initial study site (Delft Community Health Centre), an amended research proposal with a different recruitment site, namely Albow Gardens clinic, was submitted and approved in April 2013 (Addendum F). The TASK Applied Science TB research site (affiliated to SU) also granted the researcher permission to conduct the data collection on their premises at Brooklyn Chest Hospital, use their afore-mentioned equipment and utilise the services of their nursing personnel.

A detailed research proposal and application package was submitted to the City of Cape Town regulatory body. Permission was granted by the CoCT to recruit participants from Albow Gardens clinic in April 2013 (Addendum G). The facility manager/s at both the clinic and TASK site were well informed of the study and researcher intentions (See Addendum H for a copy of facility request letter).

Participants were required to give written informed consent prior to data collection (See Addendum I for informed consent forms in English, Afrikaans and isiXhosa). A short overview of the study and explanation of the consent form was shared with all participants prior to commencement of data collection. Provision was made for illiterate participants, as they were allowed to provide a thumbprint as an alternative to a signature. Consent forms were available in English, Afrikaans and isiXhosa in order to accommodate the language preference of participants. An interpreter was available on request of the participant if they were unable to understand the written consent form. Any significant modifications made to the consent form would necessitate re-signing of the forms but this was not necessary at any stage of this study. Participants were provided with personalised copies of the consent form to keep for their records.

The privacy and confidentiality of each participant was ensured by the researcher at all times during the study by making use of an anonymous approach. This was achieved as participant names were not recorded, although each person was issued with a unique identification number for the duration of the study (i.e. a randomly allocated facility folder number). This identification number was used on all data capturing sheets, NHLS forms/phlebotomy samples and Excel spreadsheets. In addition, data was securely stored by the researcher and confidentiality of information was thus ensured. The researcher was the sole person who had access to this privileged information.

Participants were also compensated for their transport costs and missed working hours according to standard remuneration tariffs.

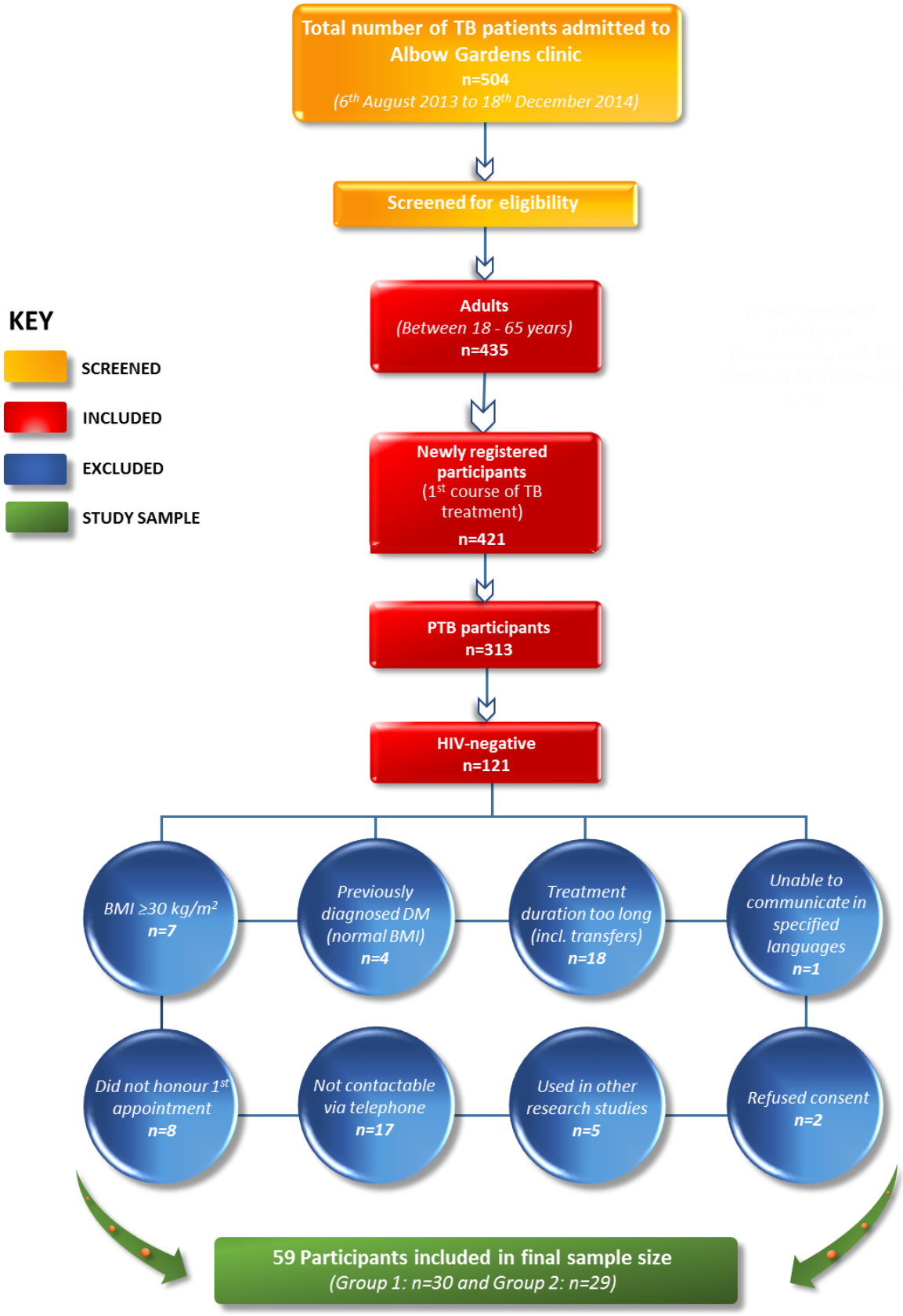
CHAPTER 3: RESULTS

3.1 STUDY POPULATION

Fifty-nine (n=59) participants were enrolled in this study out of a possible 504 admissions (11.7%) to the Albow Gardens clinic during the period August 2013 to December 2014. Figure 3.1 shows a detailed flow diagram depicting the inclusion of participants into the study.

A slight loss to follow-up occurred during this study (n=8, 13.6%). This was due to mortalities, leaving the Western Cape Province or country without notifying the clinic/researcher, as well as general defaulting of TB treatment. The data collected from the above-mentioned eight participants (who had been seen once or twice out of a possible three visits) was then transferred to Group 1 and a new participant was sourced to replace those lost to follow-up.

Unfortunately, within the last two weeks of official data collection in December 2014, the pen-ultimate follow-up participant defaulted his five-month appointment. It was decided that replacing this participant would result in an indefinite prolonging of the data collection period and research output, therefore, the follow-up group (Group 2) of participants was completed with 29, and not the originally desired 30, participants.



*Group 1 = Participants seen at baseline only; Group 2 = Participants seen at baseline, two and five months
TB = tuberculosis; PTB = pulmonary tuberculosis; HIV = human immunodeficiency virus;
BMI = body mass index; DM = diabetes mellitus

Figure 3.1: Flow diagram for inclusion of study participants

3.2 DEMOGRAPHIC DATA

3.2.1 Total Study Population (n=59)

The main demographic data of the total study population is summarised in Table 3.1. Of the fifty-nine participants included in the total study population, the majority (n=48, 81.4%) were males whilst the remaining 18.6% (n=11) were females. The mean age for all participants was 33.95 (SD 12.02) years and the greatest number of study participants fell into the 18 – 30 year age category (n=32, 54.2%), with the fewest participants (n=12, 20.3%) found in the category of 46 - 65 years. With regard to racial distribution, the majority of participants were black African (n=36, 61.0%) whereas coloured participants consisted of 28.8% of the population (n=17) and white participants the remaining 10.2% (n=6).

Table 3.1: Demographic characteristics of the total study population (n=59)

	Black African n=36 (61.0%)	**Coloured** n=17 (28.8%)	**White** n=6 (10.2%)	**TOTAL** n=59
Variable				
Gender		n		
Male	32 (88.9%)	13 (76.5%)	3 (50.0%)	48 (81.4%)
Female	4 (11.1%)	4 (23.5%)	3 (50.0%)	11 (18.6%)
Age		n		
18-30 years	22 (61.1%)	8 (47.1%)	2 (33.3%)	32 (54.2%
31-45 years	9 (25.0%)	5 (29.4%)	1 (16.7%)	15 (25.4%)
46-65 years	5 (13.9%)	4 (23.5%)	3 (50.0%)	12 (20.3%)

3.3 BASELINE ANTHROPOMETRY RESULTS

3.3.1 Baseline Mean Values

As expected, there were no significant differences seen between Groups 1 and 2 for the variables shown in Table 3.2 (anthropometry measurements), as well as the categorical variables (BMI classification, frame size, waist circumference classification, etc.). The two groups were, therefore, comparable.

Table 3.2 provides a comprehensive overview of all gender-specific anthropometrical variables measured at baseline. As the participants were never stratified and / or recruited according to gender, any possible conclusions should be interpreted with caution.

Table 3.2: Baseline gender-specific anthropometry measurements of total study population (n=59)

Variable	Unit	Male Mean (SD*)	Female Mean (SD)	p-value *	Range
Weight	**kg**	**57.70 (9.06)**	**48.14 (7.18)**	**<0.01**	**32.80-83.00**
Height	**m**	**1.72 (0.07)**	**1.56 (0.06)**	**<0.01**	**1.49-1.88**
BMI	kg/m^2	19.49 (2.46)	19.73 (3.11)	0.77	14.58-26.38
Waist circumference	cm	71.0 (7.70)	71.0 (8.04)	0.84	58.80-99.20
Hip circumference	cm	84.28 (6.50)	86.45 (9.90)	0.15	64.80-102.70
Waist:hip ratio	-	0.84 (0.07)	0.83 (0.08)	0.56	0.72-1.11
Elbow width	**cm**	**6.80 (0.36)**	**5.93 (0.23)**	**<0.01**	**5.70-7.60**
Biceps skinfold	**mm**	**2.69 (0.87)**	**4.91 (2.26)**	**<0.01**	**1.60-8.00**
Triceps skinfold	**mm**	**5.76 (2.73)**	**13.90 (6.02)**	**<0.01**	**2.90-22.20**
Subscapular skinfold	**mm**	**7.16 (2.70)**	**9.54 (4.37)**	**0.04**	**3.60-19.00**
Suprailiac skinfold	**mm**	**5.07 (3.55)**	**7.48 (3.63)**	**0.01**	**2.60-20.90**
Sum of skinfolds	**mm**	**20.69 (9.18)**	**35.83 (14.59)**	**<0.01**	**10.90-66.10**
Body density	**D**	**1.07 (0.01)**	**1.05 (0.01)**	**<0.01**	**1.02-1.09**
Fat mass	**kg**	**5.99 (3.78)**	**11.48 (4.49)**	**<0.01**	**1.64-21.84**
Fat free mass	**kg**	**51.71 (7.12)**	**36.66 (4.41)**	**<0.01**	**27.00-76.89**
Percentage body fat	**%**	**9.99 (4.96)**	**23.31 (6.56)**	**<0.01**	**3.27-33.12**

** Mann-Whitney U Test*

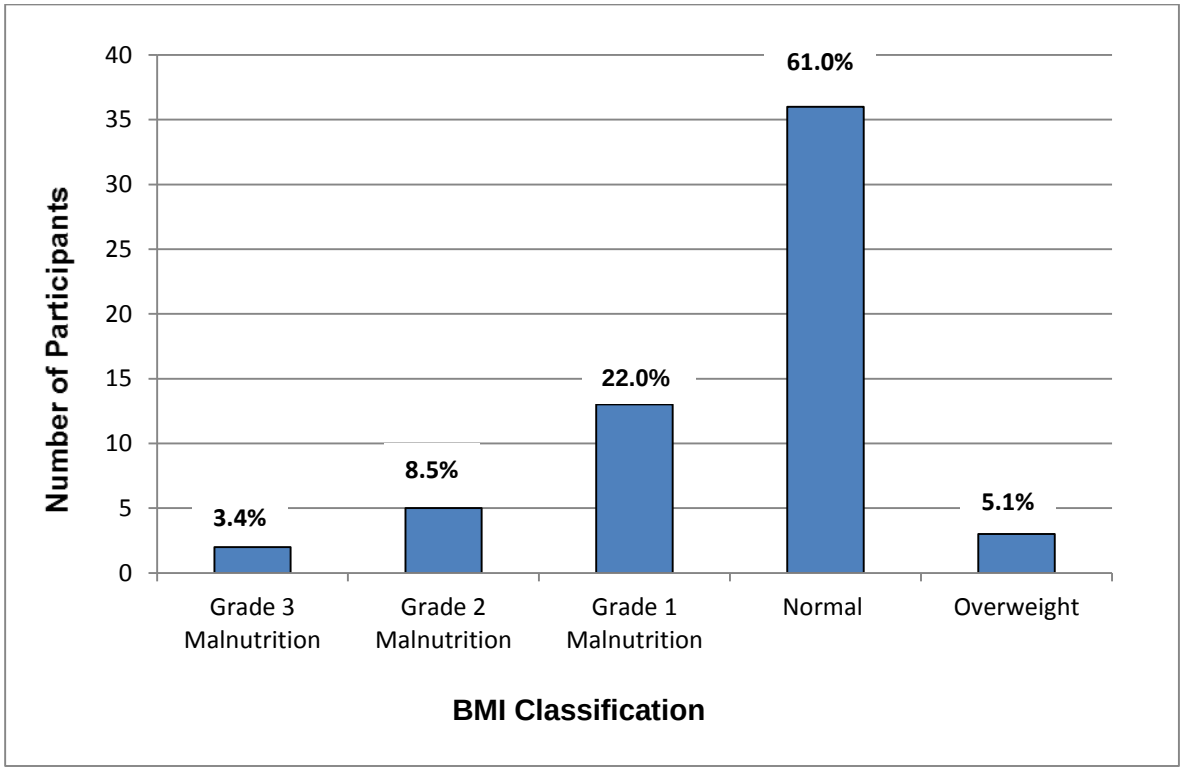
SD = standard deviation; BMI = body mass index

Bold and shaded variables indicate statistical significance

Upon consideration of Table 3.2 above, the majority of anthropometrical variables seem to differ significantly when assessing for the effect of gender. The only variables that showed no difference when factoring in gender were the BMI, waist circumference, hip circumference and the waist:hip ratio. Males displayed significantly higher measurements for weight, height, elbow width, body density and fat free mass, whereas their female counterparts showed significantly higher values for all four skinfold measurements, sum of skinfolds, fat mass and percentage body fat.

3.3.2 BMI Classification

The various BMI classifications of the total study population (n=59) are reflected in Figure 3.2. The figure shows that the majority of participants (n=36, 61.0%) enrolled in the study at baseline had a BMI in the normal range (18.50 - 24.99 kg/m^2) according to WHO cut-off points. Thirty-three point nine percent of the participants (n=20) had a BMI of <18.5 kg/m^2 at baseline. Although there were three participants (5.1%) who fell into the overweight BMI group (≥25 kg/m^2), this was in line with the inclusion criteria of the study which excluded obese participants (BMI ≥ 30kg/m^2) from recruitment.



BMI = body mass index

Figure 3.2: Baseline BMI classification of total study population (n=59)

3.3.3 Waist Circumference

Of the total male study population, the majority (n=47, 97.9%) had a waist circumference that was classified as normal. The majority of female patients also had a normal waist circumference value (n=9, 81.8%). This data can be seen in Table 3.3. A normal waist circumference for males is <94 cm, whilst for females it is <80 cm.

Table 3.3: Classification of baseline waist circumference cut-off points according to gender

Waist circumference (Males)	*Total n*	n (%)	Waist circumference (Females)	*Total n*	n (%)
<94 cm	*48*	n=47 (97.9%)	<80 cm	*11*	n=9 (81.8%)
>94 cm	*48*	n=1 (2.1%)	>80 cm	*11*	n=2 (18.2%)
≥102 cm	*48*	n=0 (0%)	>88 cm	*11*	n=0 (0%)

3.3.4 Waist:hip Ratio (WHR)

Of the male participants in the total study population, the majority (n=35, 72.9%) had a normal WHR, whilst the female population showed a higher prevalence of an increased WHR (n=5, 45.5%). Table 3.4 depicts these values, where a normal WHR for males is <0.90, whereas for females it is <0.85.

Table 3.4: Classification of baseline waist:hip ratio cut-off points according to gender

WHR (Males)	*Total n*	n (%)	WHR (Females)	*Total n*	n (%)
<0.90	*48*	n=35 (77.1%)	<0.85	*11*	n=6 (54.5%)
≥0.90	*48*	n=13 (22.9%)	≥0.85	*11*	n=5 (45.5%)

WHR = waist:hip ratio

3.3.5 Frame Size

The majority of participants in the total study population (n=35, 59.3%) were classified as having a medium frame size, whilst the remainder (n=24, 40.7%) had a small frame size. There were no participants with a large frame size in the study population.

3.3.6 Interpretation of Skinfolds

Considering the actual skinfold measurements for triceps, subscapular and sum of triceps and subscapular skinfolds, as well as percentile distributions of the above, the skinfolds were interpreted in the following way at baseline: The majority of participants fell into the 'lean' category for interpretation of triceps skinfold (n=24, 40.7%), subscapular skinfold (n=31, 52.5%) and sum of triceps and subscapular skinfolds (n=28, 47.5%), followed by either the 'below average' or 'average' categories.

3.3.7 Interpretation of Percentage (%) Body Fat

The majority of study participants (n=30, 50.8%) fell into the 'slim' category when considering their body fat percentages. 'Underweight' participants were the second most frequent occurring category (n=20, 33.9%), whereas only one participant fell into the 'excessive body fat' category (1.7%). These results are shown in Figure 3.3 below.

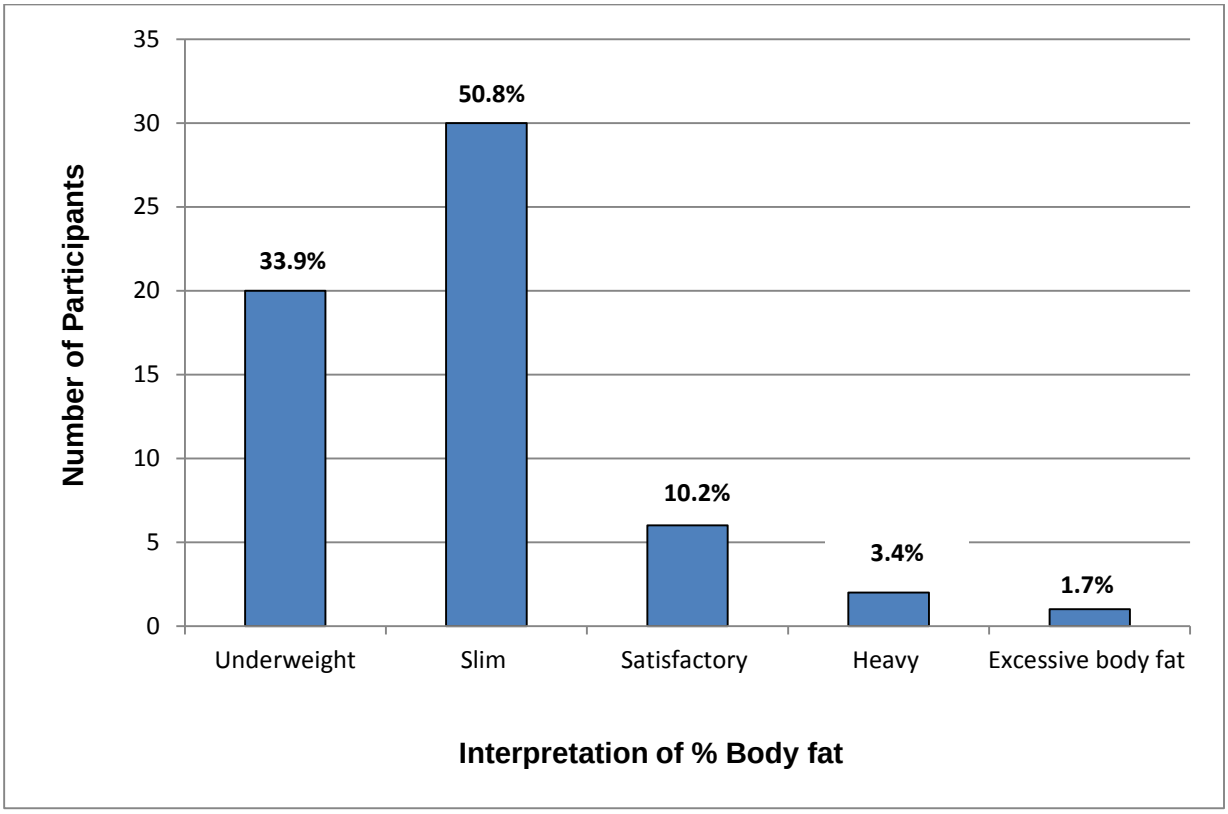


Figure 3.3: Baseline interpretation of % body fat of total study population (n=59)

If comparing the interpretation of percentage body fat at baseline between genders, males presented with 56.3% (n=27) in the 'slim' category, followed by 33.3% (n=16) in the 'underweight category'. Females showed the greatest number of participants falling into the 'underweight' category (n=4, 36.4%).

3.3.8 Comparison of Baseline Anthropometrical Variables with Demographic Data

Please note that the groups of all demographic variables (gender, race and age) were compared with least square means (LS means), which considers the differing sample sizes of groups. This is ensured by using the mean square error (MSE) as the measure of error variance, and not the variance of the individual groups.

As was true for gender, any findings generated from other demographic variables such as age and race, should also be interpreted with caution, given the lack of stratification in the study.

There were no significant differences between any of the baseline anthropometrical variables when compared with the race of the participants. With regard to the effect of age on the baseline anthropometrical variables, the only significant variables are indicated in Table 3.5.

Table 3.5: Comparison of baseline anthropometrical variables with age (n=59)

Variable (Baseline)	Unit	r-value	p-value *
Waist circumference	**cm**	**0.27**	**0.04**
Waist:hip ratio	**-**	**0.35**	**0.01**
Body density	**D**	**-0.44**	**0.00**
Fat mass	**kg**	**0.36**	**0.01**
Percentage body fat	**%**	**0.44**	**0.00**

** Spearman rank order correlation*

BMI = body mass index

Bold and shaded variables indicate statistical significance

When considering Table 3.5 above, the following variables showed a correlation with age of the participants: waist circumference, waist:hip ratio, body density, fat mass and percentage body fat. Of these variables, only body density showed a negative correlation with age, whilst the others showed a positive correlation.

3.4 FOLLOW-UP ANTHROPOMETRY RESULTS

Follow-up data refers to results from participants in Group 2 that were seen at baseline, and followed up two and five months thereafter. All measurements were done on this group and the sample size was, twenty-nine (n=29), for all variables.

A repeated measures ANOVA, with the compound symmetry assumption on the correlation structure over time, was mostly used to compare all the biochemical variables of the participants over the three periods of measurement. Please note that parametric methods (ANOVA) were used for the majority of data analyses, and confirmed with the non-parametric test if necessary. Therefore, if both parametric and non-parametric tests were performed, the latter would be used purely for confirmatory purposes and not as the initial test. If the ANOVA was not used, the alternative statistical test will be stated. This is true for any results presented in this chapter.

As is shown in Table 3.6, the null hypothesis of equality of means was rejected for all of the anthropometrical variables, except for the biceps skinfold as no significant differences were seen over time for this sole variable. The p-values shown in Table 3.6 indicate a hypothesis test to determine whether the mean values at baseline, two months and five months are the same. The bold and shaded variables indicate that the hypothesis of equal means is rejected (as given by a p-value of <0.05) and the three mean measurements differ significantly. Bonferroni multiple comparisons procedures were duly performed to assess where the differences occurred.

Table 3.6: Changes in anthropometrical variables over time in Group 2 (n=29)

Variable	Unit	ANOVA (F-test)	Baseline Mean (SD)	Two months Mean (SD)	Five months Mean (SD)	p-value
Weight	**kg**	**F(2,56) = 39.51**	**56.83 (10.66)**	**59.76 (11.65)**	**61.37 (11.26)**	**<0.001**
BMI	**kg/m^2**	**F(2,56) = 39.34**	**19.65 (2.62)**	**20.66 (2.93)**	**21.21 (2.68)**	**<0.001**
Waist circumference	**cm**	**F(2,56) = 38.44**	**70.38 (8.84)**	**72.95 (9.32)**	**75.40 (9.18)**	**<0.001**
Waist:hip ratio	**-**	**F(2,56) = 36.29**	**0.83 (0.08)**	**0.85 (0.09)**	**0.88 (0.09)**	**<0.001**
Biceps skinfold	mm	F(2,56) = 1.81	3.16 (1.45)	3.36 (1.96)	3.49 (2.02)	0.174
Triceps skinfold	**mm**	**F(2,56) = 10.05**	**7.01 (4.05)**	**7.49 (4.29)**	**8.13 (4.63)**	**<0.001**
Subscapular skinfold	**mm**	**F(2,56) = 10.22**	**7.77 (2.85)**	**8.26 (2.69)**	**8.79 (3.35)**	**<0.001**
Suprailiac skinfold	**mm**	**F(2,56) = 6.81**	**6.10 (4.61)**	**7.32 (5.50)**	**7.57 (5.11)**	**0.002**
Sum of skinfolds	**mm**	**F(2,56) = 8.64**	**24.03 (11.79)**	**26.43 (13.47)**	**27.99 (14.18)**	**<0.001**
Fat mass	**kg**	**F(2,56) = 17.63**	**7.22 (4.34)**	**8.38 (4.39)**	**8.73 (4.50)**	**<0.001**
Fat free mass	**kg**	**F(2,56) = 38.11**	**49.61 (9.56)**	**51.38 (10.13)**	**52.63 (10.23)**	**<0.001**
Percentage body fat	**%**	**F(2,56) = 10.89**	**12.58 (6.53)**	**13.85 (5.96)**	**14.19 (6.31)**	**<0.001**

SD = standard deviation; BMI = body mass index

Bold and shaded variables indicate statistical significance

3.4.1 Weight

The mean weights of the participants increased over time and a Bonferroni multiple comparisons procedure revealed differences between baseline and two months (p<0.001), baseline and five months (p<0.001) and two months and five months (p=0.009) as shown in Figure 3.4.

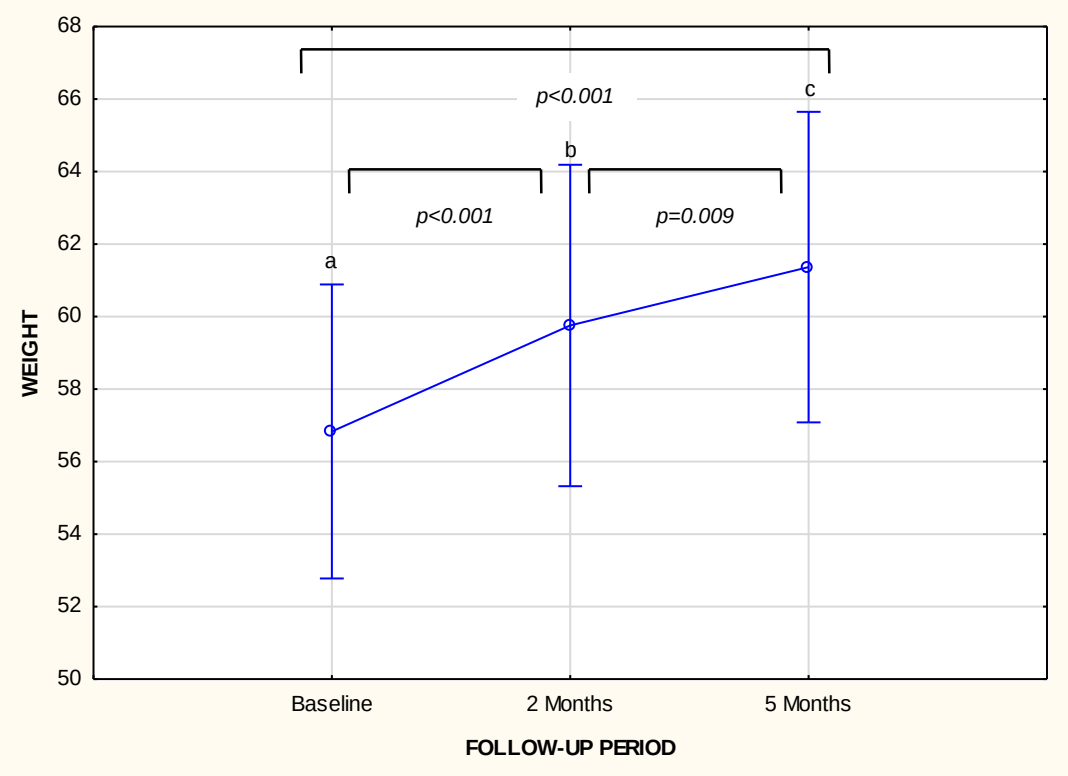
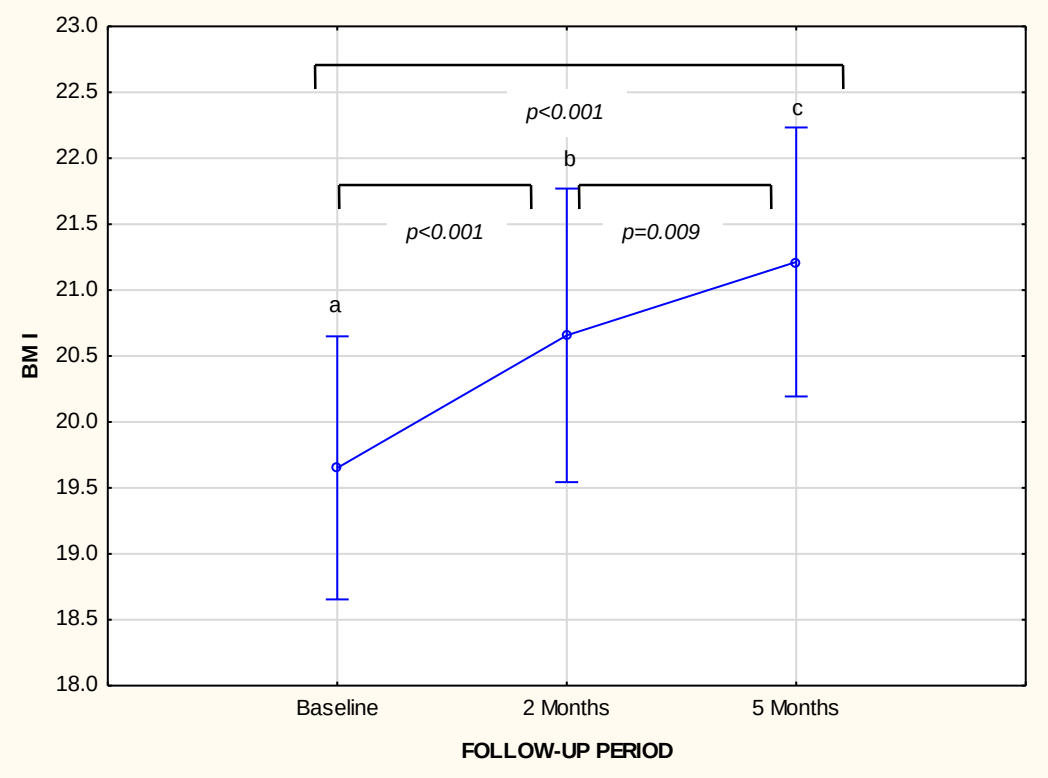


Figure 3.4: Changes in weight of participants over five-month follow-up period (n=29)

3.4.2 BMI

The mean BMI of the participants increased over the follow-up period (as seen in Figure 3.5) and a Bonferroni multiple comparisons procedure revealed that all three periods differ from one another: namely baseline and two months ($p<0.001$), baseline and five months ($p<0.001$) and two and five months ($p=0.009$).



BMI = body mass index

Figure 3.5: Changes in BMI of participants over five-month follow-up period (n=29)

3.4.3 Waist Circumference

Upon evaluating changes over time, there was a significant increase over the five month period, as shown in Figure 3.6. A Bonferroni multiple comparisons procedure revealed that there was a difference between baseline and two months ($p<0.001$), as well as between baseline and five months ($p<0.001$) and two and five months ($p<0.001$).

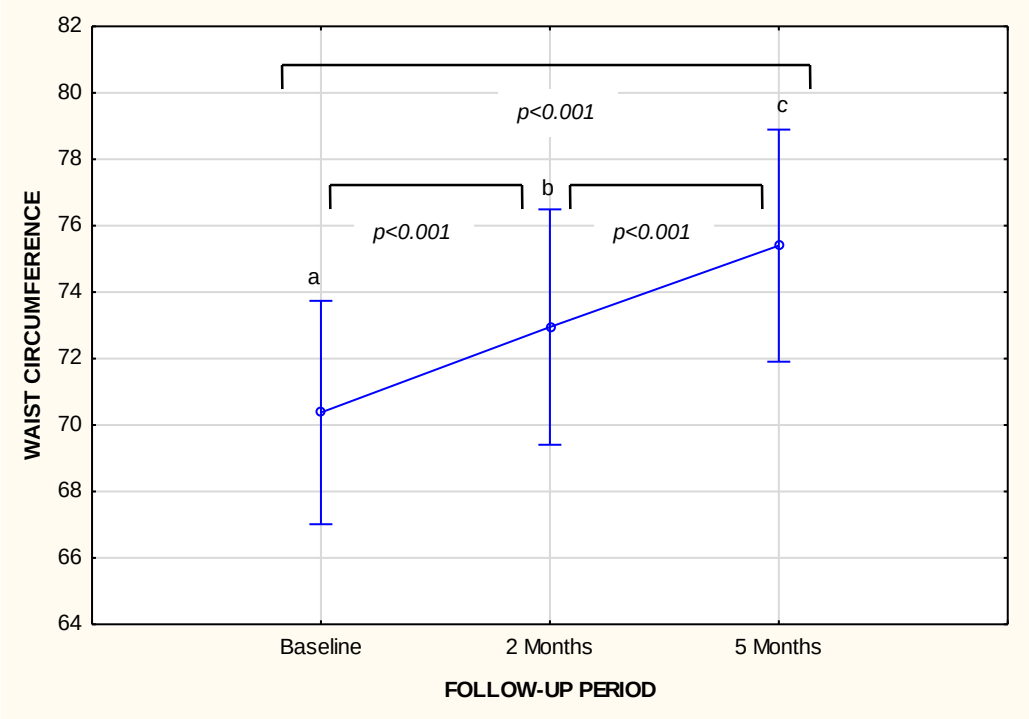


Figure 3.6: Changes in waist circumference of participants over five-month follow-up period (n=29)

3.4.4 Waist:hip Ratio (WHR)

The WHR experienced a significant increase over the five-month period (Figure 3.7) whereby a Bonferroni multiple comparisons procedure revealed that there was a difference between all three periods: namely, baseline and two months ($p<0.001$), baseline and months ($p<0.001$) and two months and five months ($p<0.001$).

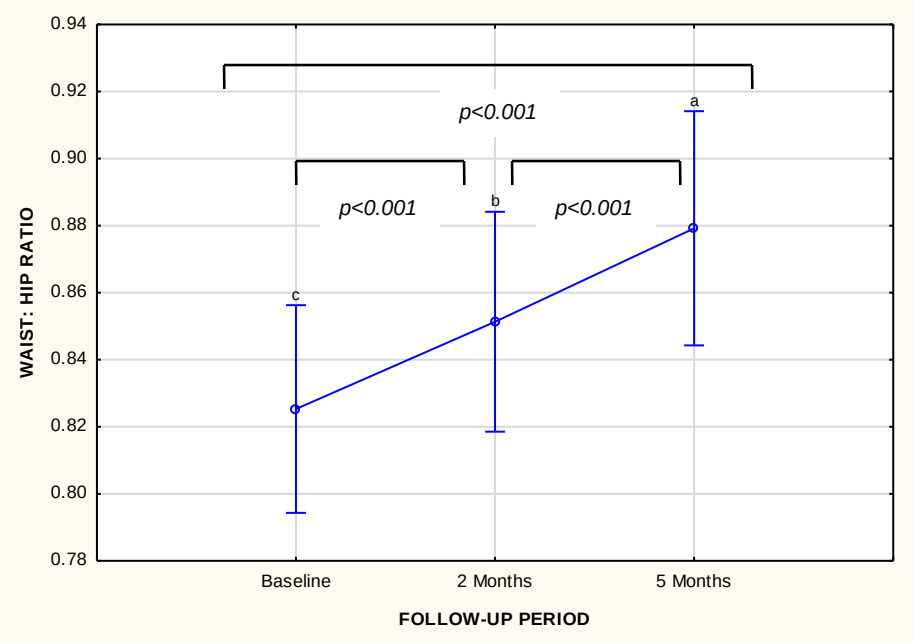


Figure 3.7: Changes in waist:hip ratio of participants over five-month follow-up period (n=29)

3.4.5 Triceps Skinfold

Despite an increase over time, a Bonferroni multiple comparisons procedure showed that there was only a difference between baseline and five months ($p<0.001$) and two to five months ($p=0.039$). There was no difference between baseline and two months ($p=0.189$). These findings were confirmed by a bootstrap test, due to the residuals not being normally distributed.

3.4.6 Subscapular Skinfold

Although there was an increase in mean values over the follow-up period, a Bonferroni multiple comparisons procedure only showed a difference between baseline and five months ($p<0.001$). No difference was found between baseline and two months ($p=0.103$) or two and five months ($p=0.068$).

3.4.7 Suprailiac Skinfold

Despite an increase over time, a Bonferroni multiple comparisons procedure showed that there was only a difference between baseline and two months (p=0.018) and baseline and five months (p=0.003). No difference was found between two and five months (p=1.000).

3.4.8 Sum of Skinfolds

Figure 3.8 shows an increase in mean values over time, and a Bonferroni multiple comparisons procedure revealed that there was a difference between baseline and two months (p=0.046), as well as baseline and five months (p<0.001). There was no difference between two and five months (p=0.329).

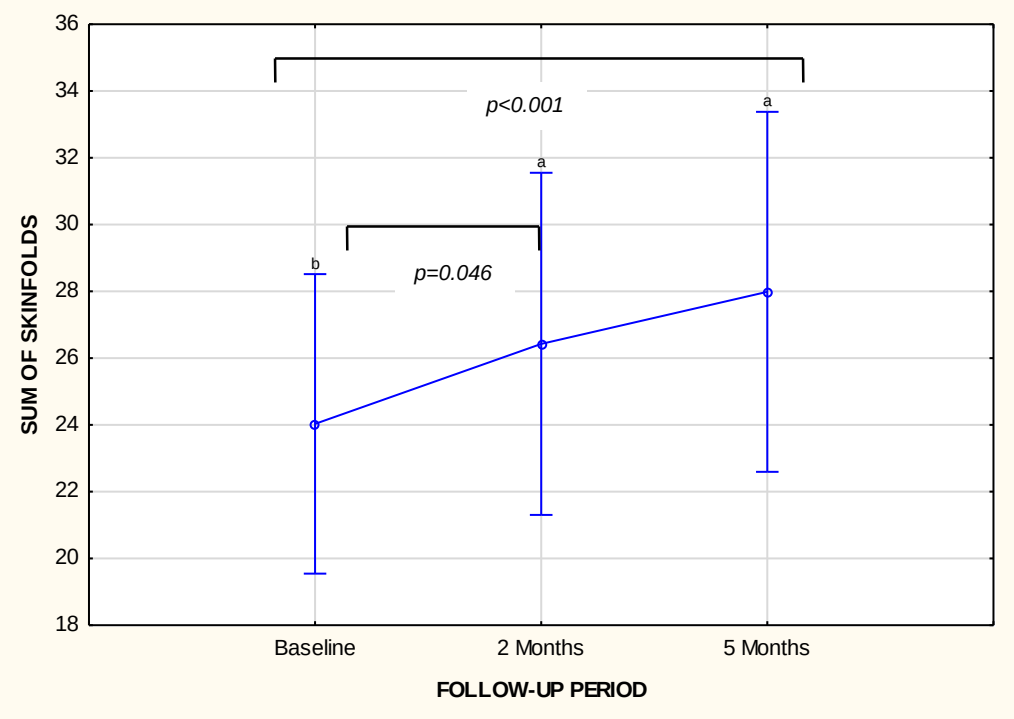


Figure 3.8: Changes in sum of skinfolds of participants over five-month follow-up period (n=29)

3.4.9 Fat Mass

There was an overall increase in the fat mass over the five-month follow-up period, whereby a Bonferroni multiple comparisons procedure showed that there was a difference in both baseline to two months ($p<0.001$) and baseline to five months ($p<0.001$). There was no difference between the two and five month mean measurements ($p=0.571$).

3.4.10 Fat Free Mass

There was an increasing trend for the measurement over all follow-up periods (Figure 3.9). A Bonferroni multiple comparisons procedure revealed that differences were found between baseline and two months ($p<0.001$), baseline and five months ($p<0.001$) and two and five months ($p=0.002$).

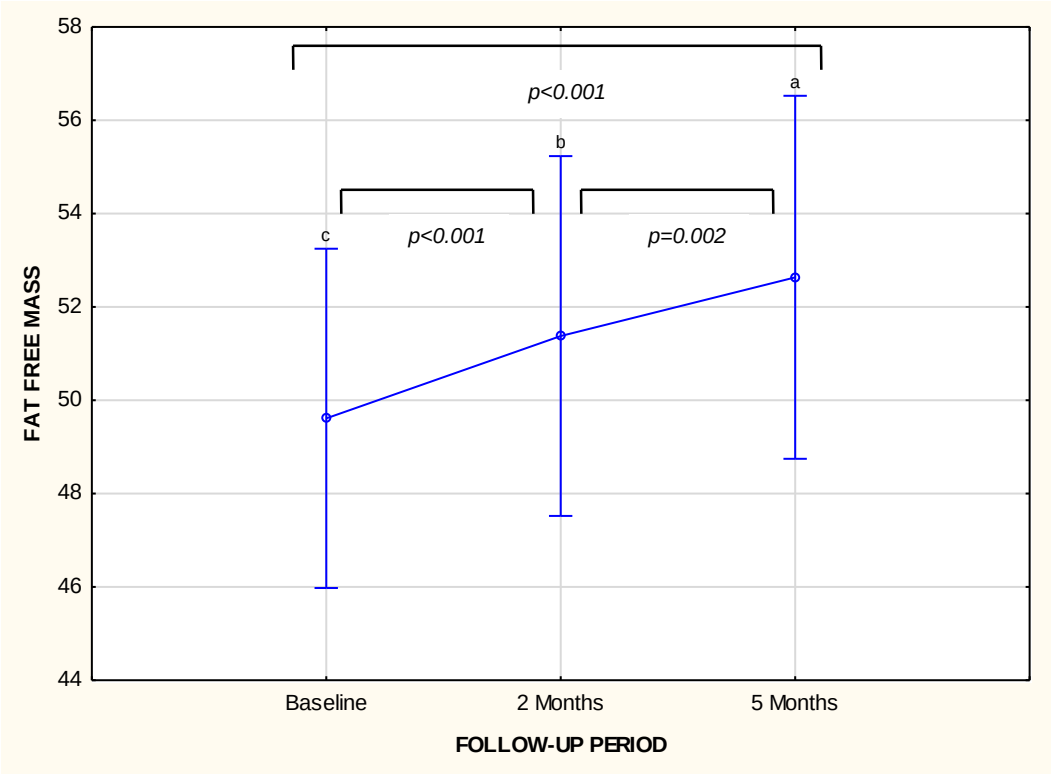


Figure 3.9: Changes in fat free mass of participants over five-month follow-up period (n=29)

3.4.11 Percentage Body Fat

Despite showing an upward trend, a Bonferroni multiple comparisons procedure only showed a difference between baseline and two months (p=0.003) and baseline and five months (p<0.001). There was no difference between the two and five month mean measurements (p=1.000).

In addition, no difference was found if comparing the effect of BMI classification on percentage body fat measurements, as the interaction between percentage body fat and BMI classification was not significant [(F (8, 48) = 1.53 (p=0.173)].

3.5 BASELINE BIOCHEMISTRY RESULTS

Table 3.7 provides a comprehensive overview of all biochemical variables measured at baseline. It is apparent from the above-mentioned table that whilst the mean CRP level was noticeably raised (indicated in Table 3.7 in bold), the mean values of albumin, white cell count and fasting glucose levels were all in the normal ranges. The mean HDL level was decreased for both genders (also indicated in bold in Table 3.7). All baseline observations discussed in this section are based on reference values provided by the NHLS (See Chapter 2.6.3.2).

Table 3.7: Baseline biochemistry measurements of total study population (n=59)

Variable	Unit	*Reference values*	Mean	SD	Range	Prevalence of abnormalities (n=59)
Albumin	g/L	*35.0-52.0*	39.32	4.35	28.00-48.00	Decreased: n=9 (**15.3%)**
White cell count	10ˆ9/L	*4.0-10.0*	8.84	3.56	3.68-19.15	Increased: n=20 (**33.9%**)
						Decreased: n=2 (**3.4%)**
Fasting glucose	mmol/ L	*4.1-5.9*	4.82	0.80	3.60-8.10	Increased: n=3 (**5.1%)**
						Decreased: n=5 (**8.5%)**
Fasting insulin	mU/L	*3.0-25.0*	11.37	19.20	0.90-142.30	Increased: n=4 (**6.8%)**
						Decreased: n=6 (**10.2%)**
CRP	mg/L	*0.0-10.0*	**60.18**	50.92	3.00-202.00	Increased: n=50 (**84.7%)**
Total cholesterol	mmol/ L	*≤5.0*	3.47	0.90	1.70-6.20	Increased: n=2 (**3.4%)**
Triglycerides*	mmol/ L	*>1.7*	0.89	0.33	0.40-2.10	Increased: n=1 (**1.7%)**
HDL-cholesterol*	mmol/ L	*>1.0 (Males)*	Total: 0.97	Total: 0.32		Total: Decreased: n=41 (**69.5%)**
			Male: 0.94	*Male: 0.88*	*0.30-1.70*	

Variable	Unit	*Reference values*	Mean	SD	Range	Prevalence of abnormalities (n=59)
HDL-cholesterol*	mmol/L	*>1.2 (Females)*	***Female: 1.14***	*Female: 0.88*	*0.60–1.50*	
LDL-cholesterol*	mmol/L	*<3.0*	2.08	0.70	0.40-4.60	Increased: n=5 (**8.5%)**

SD = standard deviation; CRP = C-reactive protein; HDL = high density lipoprotein; LDL = low density lipoprotein
**Category 2 risk (see Table 2.9 for classification)*

Upon consideration of Table 3.7 above, the most noteworthy of biochemical results show that the majority of participants (n=50, 84.7%) had raised CRP levels at baseline, whilst 69.5% (n=41) of participants had a lowered HDL-cholesterol level. Thirty-three point nine percent (n=20) of participants presented with an increased white cell count at baseline despite the mean value falling in the normal range.

There appeared to be noticeably raised values for both fasting glucose and fasting insulin levels when considering the upper range of the study population (indicated in the shaded blocks in Table 3.7). Although some of these values far exceed the normal reference values, they were captured correctly and were not erroneous outliers.

3.5.1 Comparison of Baseline Biochemical Variables with Demographic Data

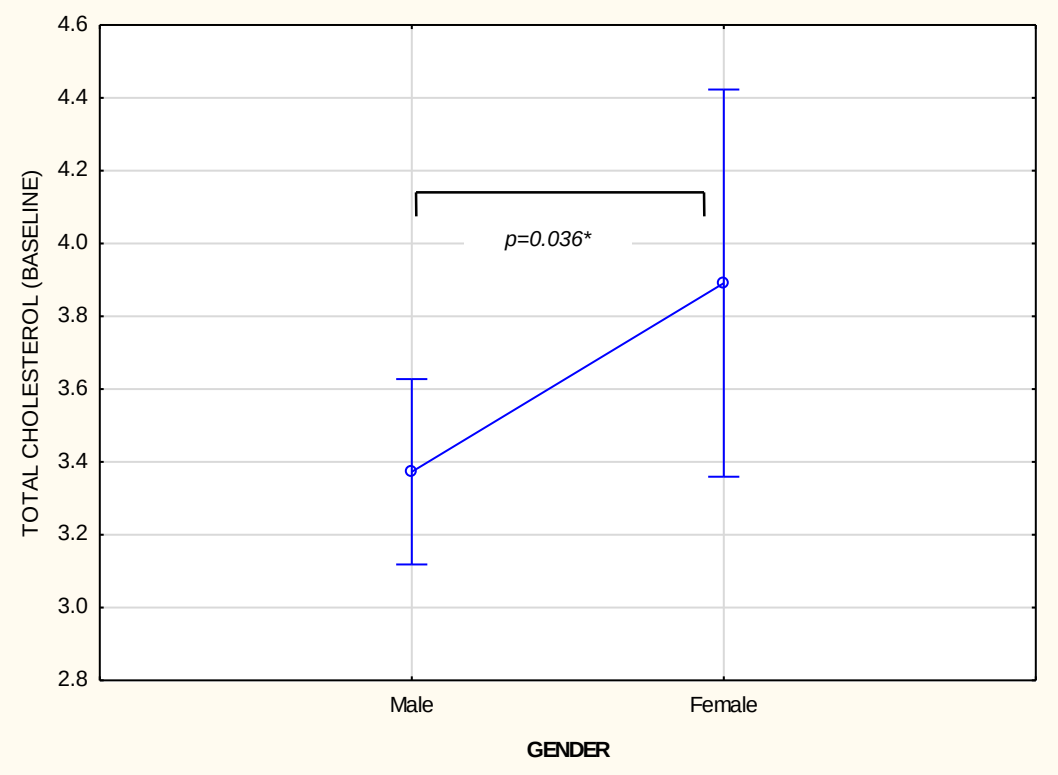
Once again, it is important to note that as the participants were not stratified according to the above demographics, any possible conclusions should be interpreted with caution. Any significant differences between biochemical variables and demographic data (gender, race or age) are discussed below.

No significant results were found between any of the demographic variables and biochemical measures studied although the LDL-cholesterol (p=0.050, Mann-Whitney U test) seemed to mimic the findings of the total cholesterol when compared with gender. There were no further notable findings with regard to either race or age and the remaining biochemical findings.

3.5.1.1 Total cholesterol - gender

When evaluating the effect of gender on baseline biochemical values, it was apparent that only the total cholesterol displayed a significant difference between males and females [Males: 3.37mg/L (0.88); Females: 3.89mg/L (0.88); p=0.036; Mann-Whitney U-test). Figure 3.10 below depicts the difference

between genders, with females showing a significantly higher total cholesterol value than their male counterparts.



** Mann-Whitney U-test*

Figure 3.10: Difference between total cholesterol values (baseline) vs. gender (n=59)

3.5.1.2 Total cholesterol – race

With regard to the effect of race on total cholesterol, Figure 3.11 depicts a significant difference between the black African and coloured groups (p=0.038) and black African and white groups (p=0.014). There was no difference between the coloured and white groups (p=0.784). This was documented with a Bonferroni multiple comparisons procedure. The white population group displays the highest total cholesterol value, whilst the black African group has the lowest [African: 3.18mg/L (0.77); Coloured: 3.81 (0.95); White (4.25 (0.79); p=0.021; Kruskal-Wallis).

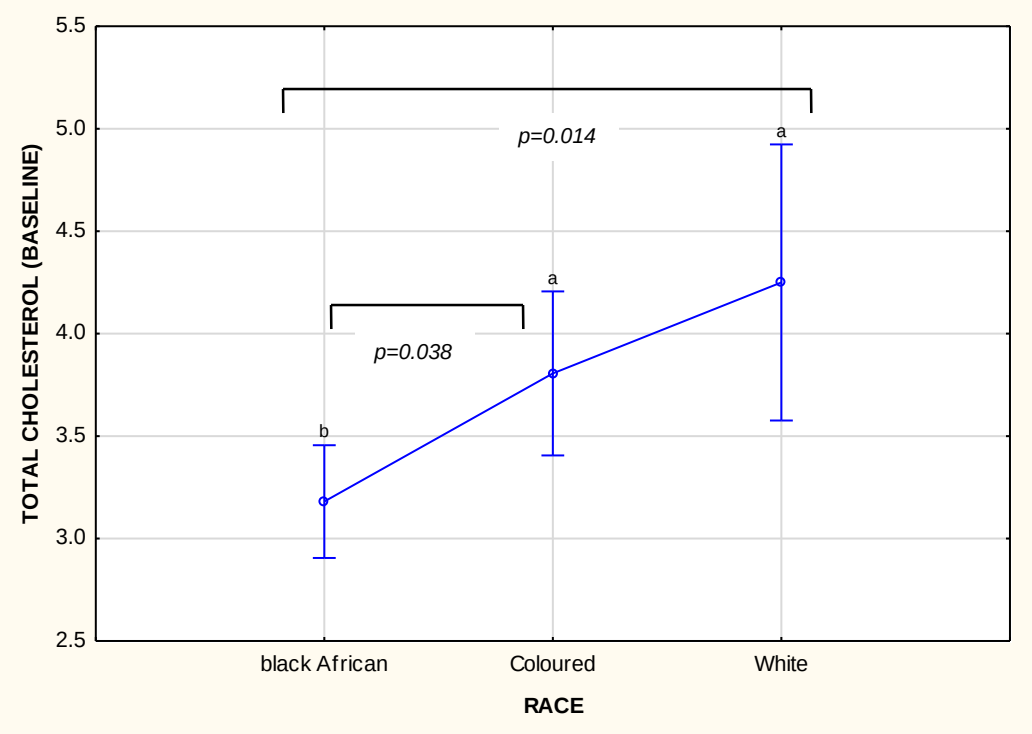
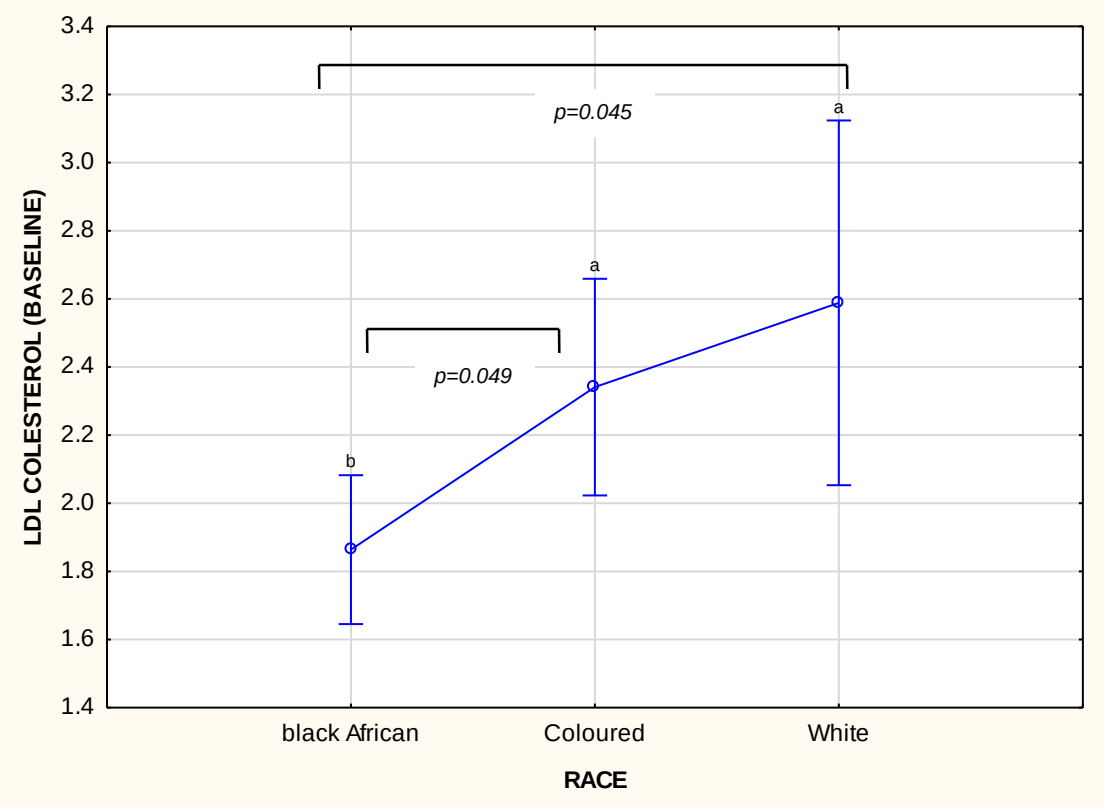


Figure 3.11: Difference between total cholesterol values (baseline) vs. race (n=59)

3.5.1.3 LDL-cholesterol - race

The baseline LDL-cholesterol of the total study population also showed significant differences when evaluating the impact of race (Figure 3.12). Once again the white population group showed the highest LDL levels, while the black African group displayed the lowest levels [African: 1.86 mmol/L (0.61); coloured: 2.34 (0.77); white (2.59 (0.56); p=0.021; Kruskal-Wallis). The LDL-cholesterol followed a similar trend as the total cholesterol, with a Bonferroni multiple comparisons procedure showing a significant difference between the black African and coloured group (p=0.049) and black African and white group (p=0.045), but no difference between the coloured and white group (p=1.000).

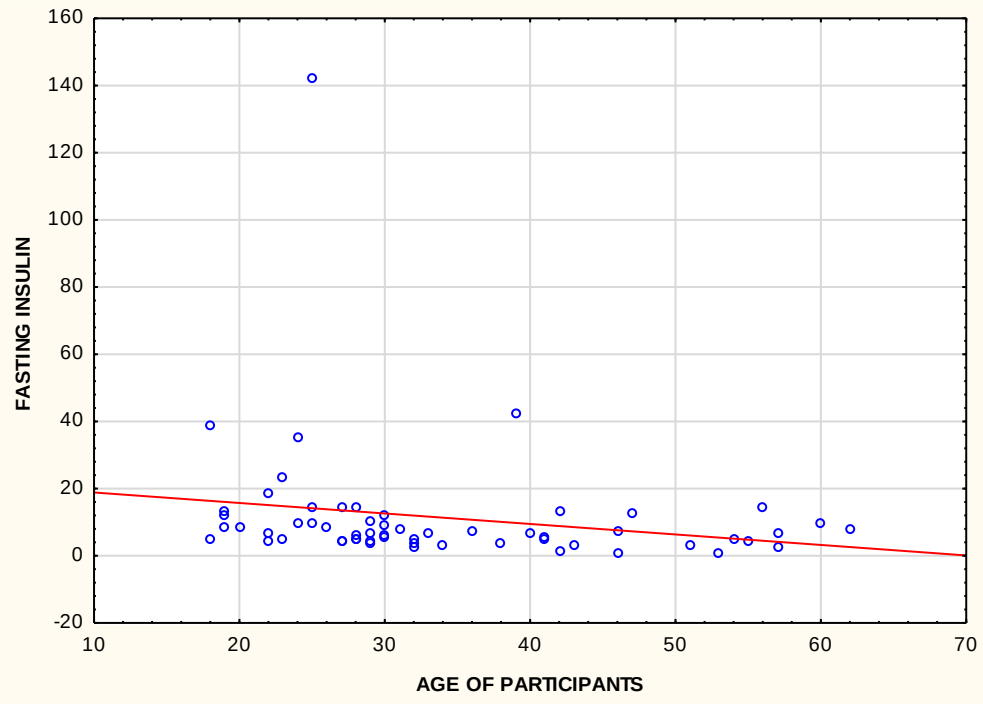


LDL = Low Density Lipoprotein

Figure 3.12: Difference between LDL-cholesterol values (baseline) vs. race (n=59)

3.5.1.4 Fasting insulin - age

Only the fasting insulin value showed any correlation with age when considering the baseline biochemical measurements (r= -0.37; p=0.004, Spearman rank order correlation). This variable showed a negative correlation with age. Therefore, as the age of the participants increased, the fasting insulin decreased, which is shown in Figure 3.13.



r= -0.37; p=0.004, Spearman Rank Order Correlation

Figure 3.13: Correlation of baseline fasting insulin with age of participants (n=59)

The presence of a much increased single value indicated in Figure 3.13 (fasting insulin value of 142.3 mU/L) is noted, although this is not an erroneous value (Refer to Chapter 3.11: Metabolic Syndrome Investigation).

3.6 FOLLOW-UP BIOCHEMISTRY RESULTS

Statistical analysis of follow-up biochemical data was performed similarly to the anthropometrical follow-up data with repeated measures ANOVA. The p-values shown in Table 3.8 also indicate a hypothesis test to determine whether the mean values at baseline, two months and five months are the same. The shaded variables indicate that the hypothesis of equal means is rejected (as given by a p-value of <0.05) and the three mean measurements differ significantly.

As is shown in Table 3.8, the null hypothesis of equality of means was rejected for the following biochemical variables: albumin, CRP, total cholesterol, HDL-cholesterol, LDL-cholesterol and white cell count, as significant changes were documented among these variables.

Table 3.8: Changes in biochemical variables over time in Group 2 (n=29)

Variable	Unit	ANOVA (F-test)	Baseline Mean (SD)	Two months Mean (SD)	Five months Mean (SD)	p-value
Albumin	**g/L**	**F(2,56) = 15.36**	**39.97 (4.06)**	**42.55 (3.90)**	**43.41 (3.77)**	**<0.001**
Fasting glucose	mmol/L	F(2,56) = 2.11	4.78 (0.61)	4.83 (0.58)	4.57 (0.50)	0.131
CRP	**mmol/L**	**F(2,56) = 21.63**	**56.72 (50.83)**	**27.54 (32.11)**	**12.48 (15.07)**	**<0.001**
Fasting insulin	mU/l	F(2,56) = 1.16	14.62 (25.98)	11.91 (15.81)	8.99 (6.77)	0.320
Total cholesterol	**mg/L**	**F(2,56) = 10.43**	**3.55 (0.95)**	**4.20 (1.06)**	**3.94 (0.84)**	**<0.001**
Triglycerides	mmol/L	F(2,56) = 0.85	0.85 (0.30)	0.93 (0.38)	0.89 (0.38)	0.432
HDL-cholesterol	**mmol/L**	**F(2,56) = 14.50**	**0.99 (0.30)**	**1.30 (0.46)**	**1.31 (0.38)**	**<0.001**
LDL-cholesterol	**mmol/L**	**F(2,56) = 4.97**	**2.17 (0.75)**	**2.48 (0.87)**	**2.22 (0.70)**	**0.010**
White cell count	**10^9/l**	**F(2,56) = 16.30**	**8.74 (4.18)**	**6.98 (2.91)**	**5.92 (2.01)**	**<0.001**

SD = standard deviation; CRP = C-reactive protein; HDL = high density lipoprotein; LDL = low density lipoprotein

Bold and shaded variables indicate statistical significance

3.6.1 Albumin

The mean albumin level of the participants increased and a Bonferroni multiple comparisons procedure revealed that baseline and two months differ (p=0.001) and baseline and five months (p<0.001), as shown in Figure 3.14. There was no difference between mean values at two and five months (p=0.567).

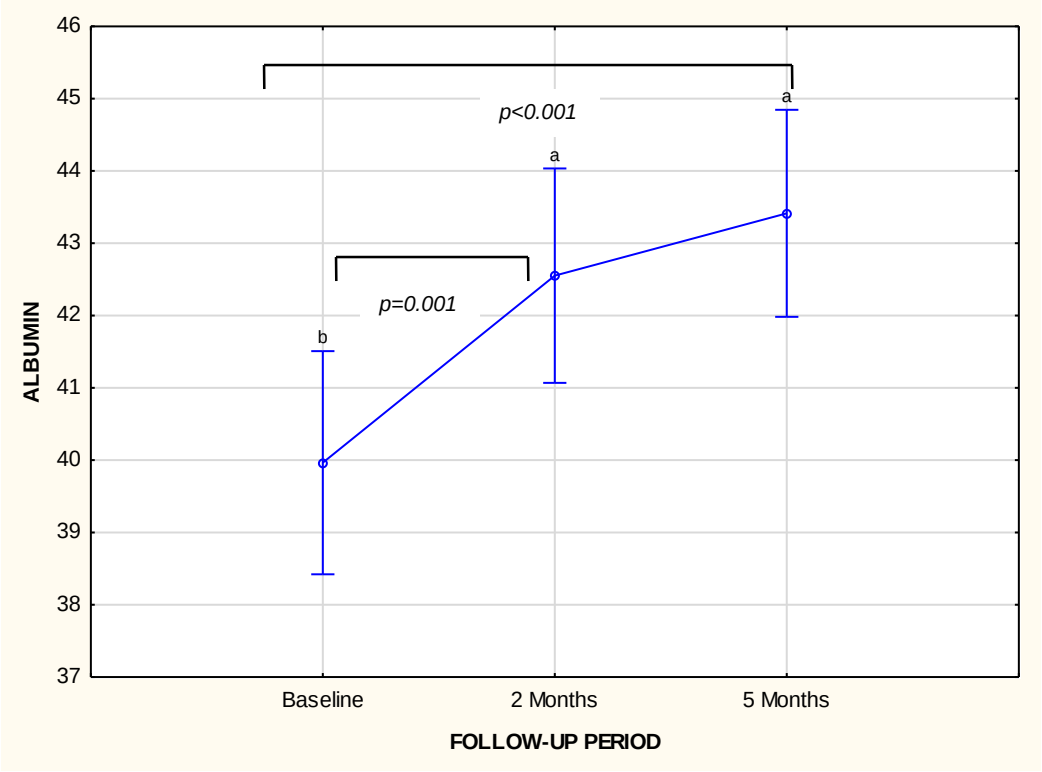


Figure 3.14: Changes in albumin of participants over five-month follow-up period (n=29)

3.6.2 White Cell Count

The mean white cell count of the participants decreased over time as shown in Figure 3.15. A Bonferroni multiple comparisons procedure showed that baseline and two months differ (p=0.002), as well as baseline and five months (p<0.001), whilst there was no difference between two and five month mean values (p=0.117).

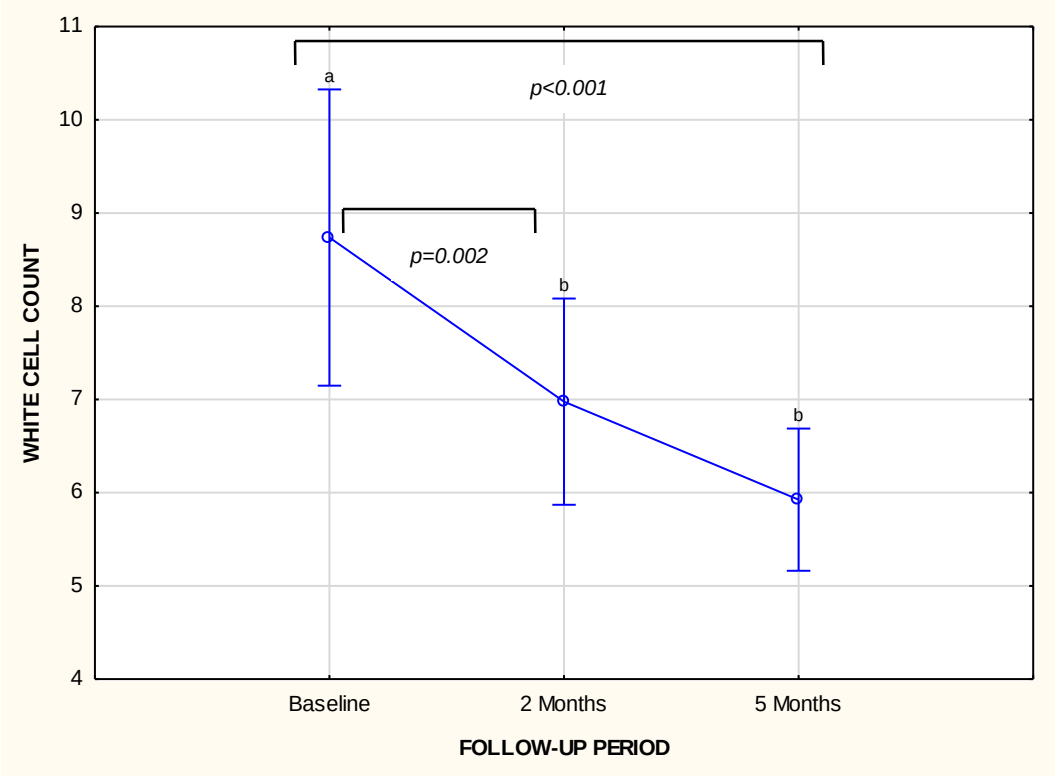
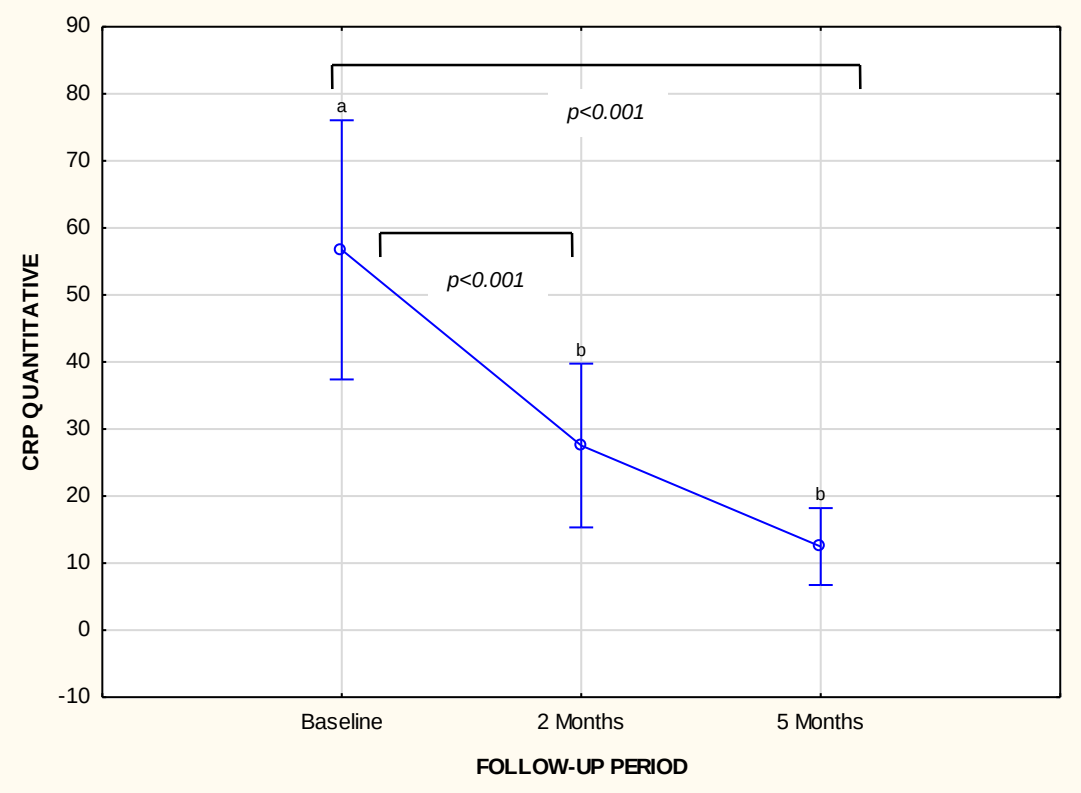


Figure 3.15: Changes in white cell count of participants over five-month follow-up period (n=29)

3.6.3 CRP

The mean measurement decreases over time, as is seen in Figure 3.16. There was a difference in mean CRP values between baseline and two months (p<0.001) and baseline and five months (p<0.001) (Bonferroni multiple comparisons procedure). There was no difference in mean CRP between two months and five months (p=0.095).



CRP = C-reactive protein

Figure 3.16: Changes in CRP of participants over five-month follow-up period (n=29)

3.6.4 Total Cholesterol

The mean measurement increases between baseline and two months, and then decreases slightly between two and five months, as shown in Figure 3.17. A Bonferroni multiple comparisons procedure revealed that there was a difference between baseline and two-month mean values ($p<0.001$) as well as between baseline and five months ($p=0.028$). No difference was found between two and five month mean values ($p=0.209$).

The LDL-cholesterol measurement followed a similar pattern to the total cholesterol measurement. The mean LDL increased between baseline and two months, but then decreased between two and five months, as seen in Figure 3.20. A Bonferroni multiple comparisons procedure revealed that there was only a difference between baseline and two month mean values ($p=0.014$), whereas no differences were found between baseline to five months or two to five months.

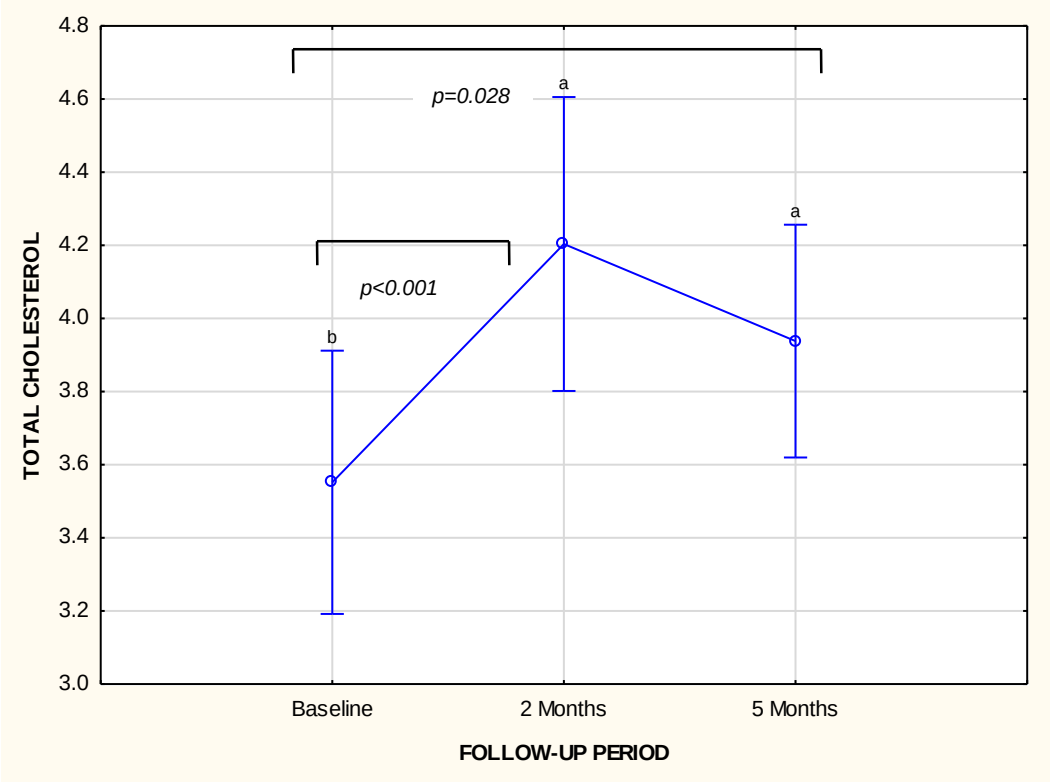
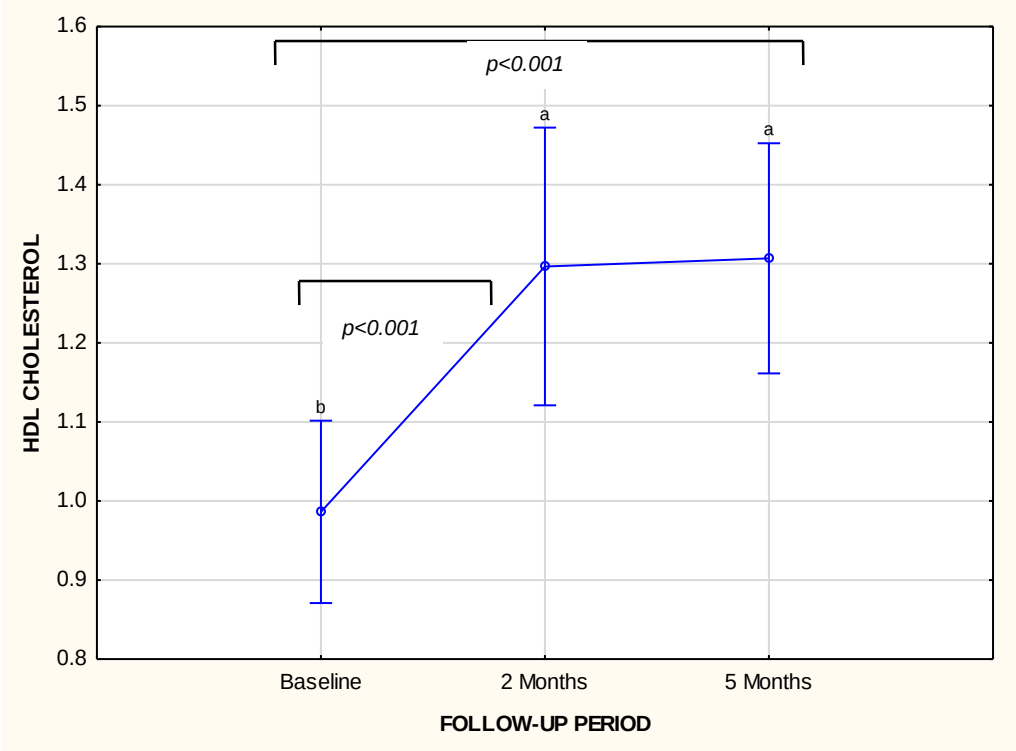


Figure 3.17: Changes in total cholesterol of participants over five-month follow-up period (n=29)

3.6.5 HDL-Cholesterol

The mean measurement increases sharply between baseline and two months, and experiences a slight increase between two and five months, as shown in Figure 3.18. There was a difference between baseline and two month mean values ($p<0.001$) as well as between baseline and five months ($p<0.001$) (Bonferroni multiple comparisons procedure). No difference was found between two and five month mean values ($p=1.000$).



HDL = high density lipoprotein

Figure 3.18: Changes in HDL-cholesterol of participants over five-month follow-up period (n=29)

3.7 BASELINE BLOOD PRESSURE RESULTS

Table 3.9 provides a comprehensive overview of blood pressure results measured at baseline.

Table 3.9: Baseline blood pressure measurements of total study population (n=59)

Variable	*Valid n*	Mean	SD	Range
Systolic Blood Pressure (mmHg)	*59*	120.73	16.53	84.00-169.00
Diastolic Blood Pressure (mmHg)	*59*	76.59	12.05	48.00-110.00

SD =standard deviation

3.7.1 Baseline Classification of Blood Pressure

According to the cut-off values for blood pressure (see classification table in Chapter 2.6.3.3), the following was observed: The majority of participants presented with a blood pressure in the normal range (<120/80 mmHg) (n=40, 67.8%), followed by 18.7% in the pre-hypertensive stage (120 – 139 / 80 – 89 mmHg). The minority of patients were classified with hypertension according to baseline readings. This information is depicted in Figure 3.19. Please note that due to the presence of a relatively small study population, blood pressure values were not classified according to age-specific categories, as these even smaller sub-samples may have affected the quality of statistical conclusions drawn.

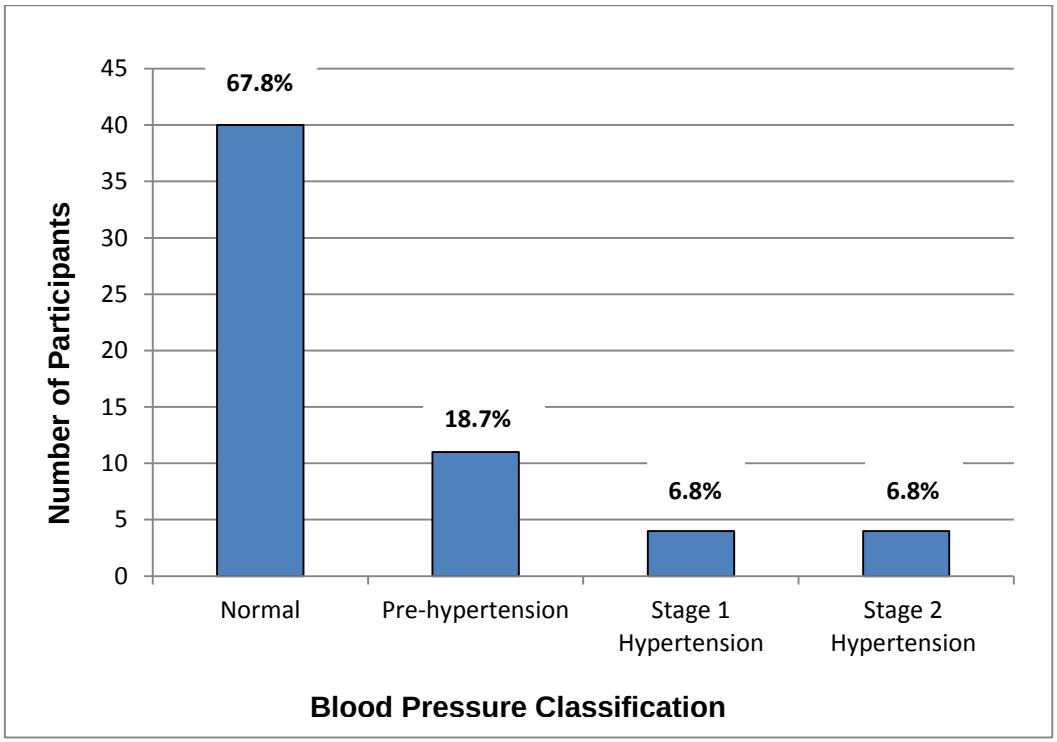


Figure 3.19: Classification of blood pressure measurements at baseline for total study population (n=59)

3.7.2 Comparison of Baseline Blood Pressure Variables with Demographic Data

Upon consideration of the effect(s) of gender, race and age on blood pressure measurements, there were no significant differences found (Table 3.10).

Table 3.10: Differences between baseline blood pressure and demographic variables (n=59)

Baseline	Gender	Race	Age
	p-value	*p-value*	*p-value*
Systolic Blood Pressure	0.599*	0.853**	0.852***
Diastolic Blood Pressure	0.586*	0.608**	0.918***

* *Mann-Whitney U test*

** *Kruskal-Wallis*

*** *Spearman rank order correlation*

3.8 FOLLOW-UP BLOOD PRESSURE RESULTS

Analysis of follow-up data was performed similarly to the anthropometrical follow-up data with repeated measures ANOVA. The p-values shown in Table 3.11 also indicate a hypothesis test to determine whether the mean values at baseline, two months and five months are the same.

Table 3.11: Changes in blood pressure variables over time in Group 2 (n=29)

Variable	Unit	ANOVA (F-test)	Baseline Mean (SD)	Two months Mean (SD)	Five months Mean (SD)	p-value
Systolic blood pressure	mmHg	F (2, 56) = 2.64	120.69 (SD15.32)	126.03 (12.31)	127.93 (17.32)	0.081
Diastolic blood pressure	mmHg	F (2, 56) = 2.45	76.00 (9.25)	80.86 (8.42)	78.76 (13.27)	0.096

SD = standard deviation

As is shown in Table 3.11, the null hypothesis of equality of means over the three repetitions was not rejected for either systolic or diastolic blood pressure, indicative of no significant difference over time. Despite the lack of significance, both systolic and diastolic blood pressure values seemed to show an upward trend over time.

3.9 BASELINE DIAGNOSTIC INSULIN RESISTANCE TESTS

Table 3.12 provides a comprehensive overview of the diagnostic IR tests measured at baseline. Both the HOMA-IR and QUICKI values were calculated as per the formulae shown in Chapter 2.6.3.4. In order to calculate these values, both the fasting glucose and fasting insulin values were needed.

Table 3.12: Baseline diagnostic IR test measurements of total study population (n=59)

Variable	Mean	SD	Range
HOMA-IR	2.72	5.74	0.17-42.37
QUICKI	0.37	0.05	0.24-0.54

SD = standard deviation; HOMA-IR = homeostasis model assessment-insulin resistance; QUICKI = quantitative insulin sensitivity check index

3.9.1 Comparison of Baseline Diagnostic Insulin Resistance Tests with Demographic Data

Table 3.13 depicts the effects of gender, race and age on the HOMA-IR and QUICKI values at baseline.

Table 3.13: Differences between baseline insulin resistance tests and demographic variables (n=59)

Baseline	Gender	Race	Age
	p-value	*p-value*	*p-value*
HOMA-IR	0.778*	0.897**	**0.014*** **
QUICKI	0.778*	0.897**	**0.014*** **

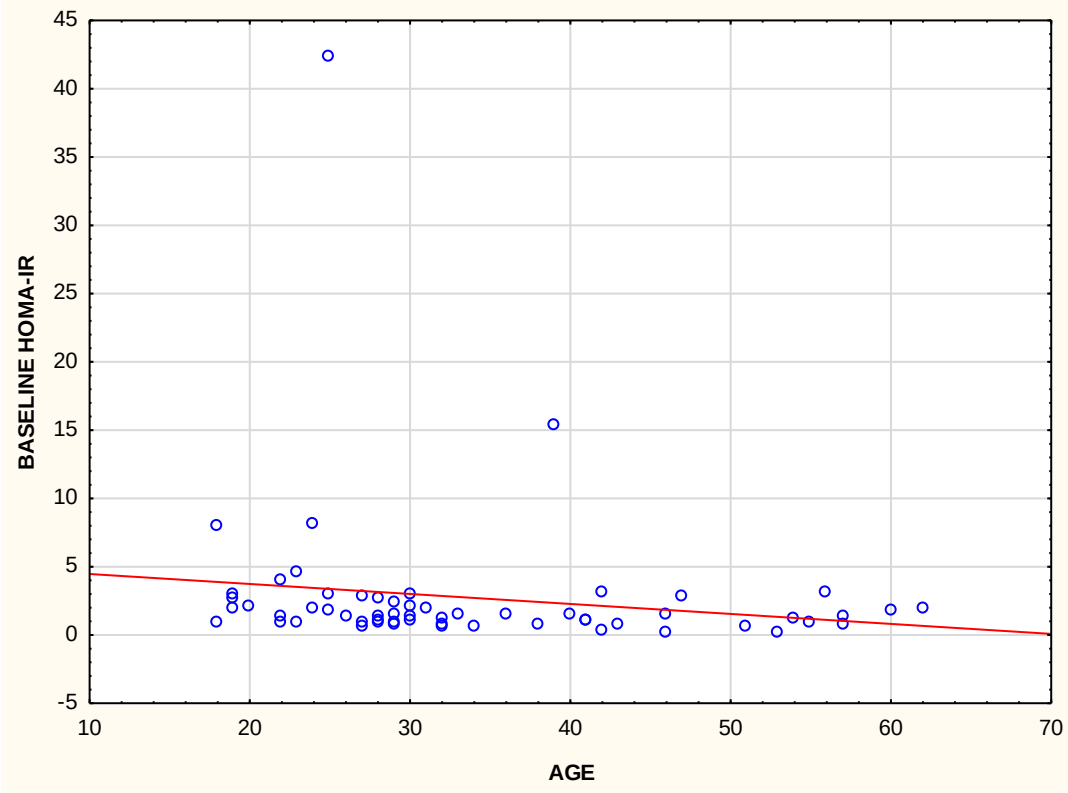
* *Mann-Whitney U test*

** *Kruskal-Wallis*

*** *Spearman rank order correlation*

Bold and shaded variables indicate statistical significance

The only significant differences found occurred between the HOMA-IR and QUICKI tests and age. This signifies that whilst HOMA-IR has a negative correlation with age, the QUICKI has a positive correlation. Therefore, as age increases the HOMA-IR decreases, whilst the QUICKI increases. The correlation between HOMA-IR and age is shown graphically in Figure 3.20 below.



r = -0.32; p=0.014; Spearman Rank Order Correlation

HOMA-IR = homeostasis model assessment – insulin resistance

Figure 3.20: Correlation of baseline HOMA-IR with age of participants (n=59)

None of the other variables showed any significant difference when comparing the effects of gender or race (Table 3.13).

3.10 FOLLOW-UP DIAGNOSTIC INSULIN RESISTANCE TESTS

Analysis of follow-up data was performed as for the anthropometrical follow-up data (Refer to Chapter 3.4 for more detail regarding statistical methods used). The p-values shown in Table 3.14 also indicate a hypothesis test to determine whether the mean values at baseline, two months and five months are the same.

Table 3.14: Changes in diagnostic IR test measurements over time in Group 2 (n=29)

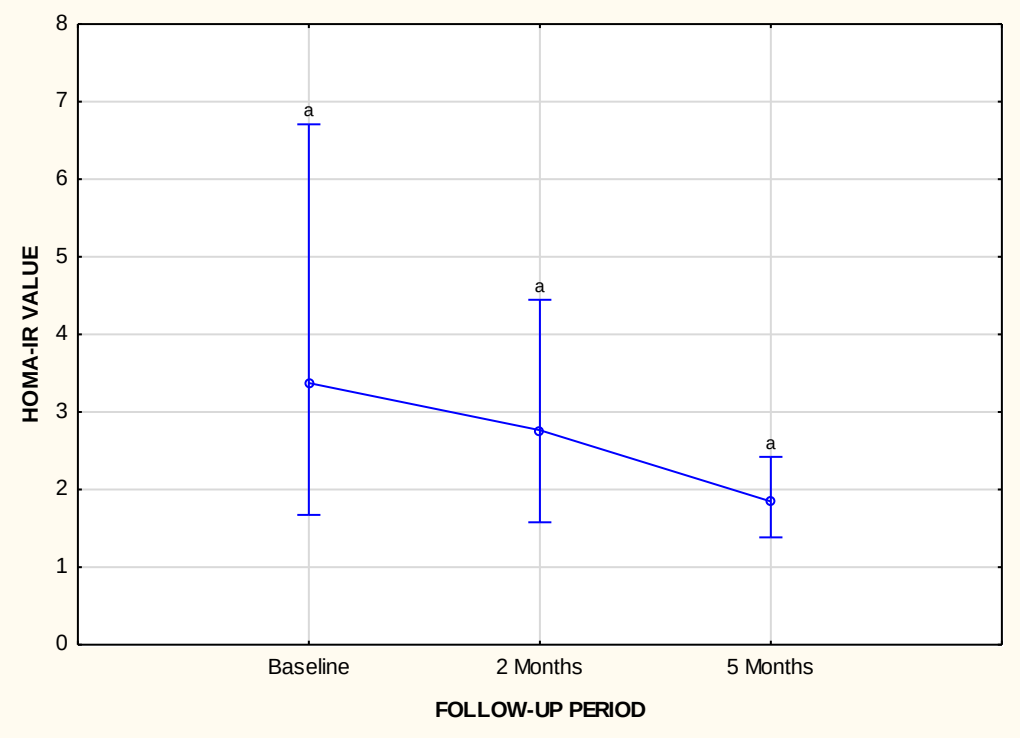
Variable	ANOVA (F-test)	Baseline Mean (SD)	Two months Mean (SD)	Five months Mean (SD)	p-value
HOMA-IR	F (2, 56) = 1.19	3.53 (7.70)	2.79 (4.35)	1.87 (1.49)	0.311
QUICKI	F (2, 56) = 0.72	0.36 (0.05)	0.36 (0.06	0.37 (0.05)	0.491

SD = standard deviation; HOMA-IR = homeostasis model assessment-insulin resistance; QUICKI = quantitative insulin sensitivity check index

As is shown in Table 3.24, the null hypothesis of equality of means over the three repetitions was not rejected for either of the diagnostic IR tests, which indicates no significant difference over time.

3.10.1 HOMA-IR

As the results were not normally distributed, a bootstrap test was performed (Figure 3.21), which confirmed that there were no significant differences present (p=0.311) between the three groups even though the HOMA-IR values experienced a slight decrease over time. No Bonferroni procedure was performed due to there being no difference in HOMA-IR means over time.



HOMA-IR = homeostasis model assessment-insulin resistance (p=0.311)

Figure 3.21: Changes in HOMA-IR values of participants over five-month follow-up period (n=29)

3.10.2 QUICKI

Similarly to the HOMA-IR findings, the QUICKI results were also not normally distributed. A bootstrap test was performed and revealed that there were no significant differences present between the three groups despite a corresponding increase in QUICKI values over time.

3.11 METABOLIC SYNDROME INVESTIGATION

Participants were also assessed according to the IDF and ATP III criteria for metabolic syndrome to investigate whether any of the study participants presented with the syndrome after study inclusion. These two frameworks were selected purely because all of the reference frameworks were investigated in the current study (See Table 1.4 for comprehensive summary of available criteria).

3.11.1 Baseline Findings

3.11.1.1 International Diabetes Federation (IDF) criteria

According to the most recent IDF criteria, in order to diagnose the metabolic syndrome, a waist circumference of more than >94 cm for males and >80 cm for females is needed.[9] If these cut-off points are applied to the total study population, there were two females (n=2) and one male (n=1) who had waist circumference's more than the cut-off's at baseline. The male was part of Group 2 seen on three occasions (male 'a'), whilst both females were part of Group 1 that were only seen once-off (females 'a' and 'b'). Table 3.13 shows that both of the females with an increased waist circumference were classified as having the metabolic syndrome according to the IDF framework.

3.11.1.2 Third Adult Treatment Panel (ATP III) criteria

According to the ATP III criteria for the identification of metabolic syndrome, ≥three (3) of the tabulated variables need to be present in an individual. Of the two individuals that were identified by the IDF criteria to have the metabolic syndrome at baseline, only one (female 'a') was identified by the ATP III criteria for the same condition (n=1, 1.7%). Female 'a' also presented with an increased HOMA-IR and decreased QUICKI at this point. Female 'b' had normal HOMA-IR and QUICKI values at baseline.

The male did not meet the criteria, which is shown in Table 3.15 (together with the females 'a' and 'b'). There were, therefore, no male participants identified with the metabolic syndrome at baseline according to both classification methods.

Table 3.15: Participants meeting IDF and ATP III criteria for metabolic syndrome at baseline

Participant	Increased WC	Increased triglycerides	Decreased HDL-cholesterol	Increased blood pressure	Increased fasting glucose
IDF Criteria					
Female 'a'	**Yes**	**No**	**Yes**	**Yes**	**Yes**
Female 'b'	**Yes**	**No**	**Yes**	**Yes**	**No**
Male 'a'	Yes	No	Yes	No	No
ATP III Criteria					
Female 'a'	**No**	**No**	**Yes**	**Yes**	**Yes**
Female 'b'	No	No	Yes	Yes	No
Male 'a'	No	No	Yes	No	No

IDF = International Diabetes Federation; ATP = Adult Treatment Panel; HDL = high density lipoprotein
'Yes' denotes the presence of the condition
'No' denotes the absence of the condition
'a' and 'b' denote different study participants (male and female)
Participants identified as having the metabolic syndrome are shown in bold (shaded area)

The IDF and ATP III criteria thus differ at baseline according to the classification of participants.

3.11.2 Follow-up Findings

For the male participant ('a') with the increased waist circumference (IDF) in Group 2, his metabolic syndrome markers over the five-month period are shown in Table 3.16.

For Male 'a', both the IDF and ATP III criteria show that he does not present with the metabolic syndrome at either baseline, two months or five months. His HOMA-IR was increased and QUICKI decreased at baseline and five months, but not at the two-month follow-up.

A second participant (Male 'b') was not identified by the IDF criteria to present with the metabolic syndrome (due to the absence of an increased waist circumference), but was however found to meet the ATP III criteria at his two-month follow-up. This was due to the presence of reduced HDL-cholesterol, raised blood pressure and increased fasting glucose level at two months (as shown in Table 3.16). He

presented with an increased HOMA-IR and decreased QUICKI at baseline and two months, but not at five-month follow-up.

Table 3.16: Male participants in Group 2:

Changes in metabolic syndrome criteria over time

Participant	Increased WC	Increased triglycerides	Decreased HDL-cholesterol	Increased blood pressure	Increased fasting glucose
IDF Criteria					
Male 'a' – Baseline	Yes	No	Yes	No	No
Male 'a' – two months	Yes	No	No	Yes	No
Male 'a'– five months	Yes	No	No	No	No
ATP III Criteria					
Male 'a' – Baseline	No	No	Yes	No	No
Male 'a' – two months	Yes	No	No	Yes	No
Male 'a'– five months	Yes	No	No	No	No
IDF Criteria					
Male 'b' – Baseline	No	No	Yes	No	Yes
Male 'b' – two months	No	No	Yes	Yes	Yes
Male 'b'– five months	No	No	No	No	No
ATP III Criteria					
Male 'b' – Baseline	No	No	Yes	No	Yes
Male 'b' – two months	**No**	**No**	**Yes**	**Yes**	**Yes**
Male 'b'– five months	No	No	No	No	No

IDF = International Diabetes Federation; ATP = Adult Treatment Panel; HDL =high density lipoprotein

'Yes' denotes the presence of the condition

'No' denotes the absence of the condition

'a' and 'b' denote different study participants (males)

Participants identified as having the metabolic syndrome are shown in bold (shaded area)

The ATP III classification differs to that of the IDF, as none of the follow-up participants were classified by the IDF as having metabolic syndrome at any stages of the data collection period.

3.12 CORRELATION BETWEEN METABOLIC SYNDROME CRITERIA AND DIAGNOSTIC INSULIN RESISTANCE TESTS

When considering the individual effect of the diagnostic criteria used in both the IDF and ATP III definitions, the correlation between each variable (waist circumference, triglycerides, HDL-cholesterol, blood pressure and fasting glucose) and diagnostic IR tests (HOMA-IR/QUICKI) was investigated. The effects at baseline regarding the total study population (n=59) are shown in Table 3.17. The effect of each variable on HOMA-IR / QUICKI values at all stages of follow-up (baseline, two months and five months) is shown in Table 3.16.

Table 3.17: Correlation between metabolic syndrome criteria and diagnostic IR tests at baseline (n=59)

Variable (at baseline)	HOMA-IR		QUICKI	
	r-value (Spearman*)	**p-value**	**r-value** (Spearman*)	**p-value**
Waist Circumference	0.13	0.34	-0.13	0.34
Triglycerides	0.14	0.30	-0.14	0.30
HDL-cholesterol	-0.19	0.16	0.19	0.16
Systolic blood pressure	0.14	0.28	-0.14	0.28
Diastolic blood pressure	0.15	0.25	-0.15	0.25
Fasting glucose**	**0.40**	**<0.01**	**-0.40**	**<0.01**

**Spearman rank correlation co-efficient*

HOMA-IR = homeostasis model assessment-insulin resistance; QUICKI = quantitative insulin sensitivity check index; HDL = high density lipoprotein

Bold and shaded variables indicate statistical significance

It is evident from the above table that the only variable to show a correlation with HOMA-IR and QUICKI values was fasting glucose (which is indicated in bold in Table 3.17).

Table 3.18 shows a similar correlation between HOMA-IR / QUICKI and fasting glucose at all three follow-up periods among participants in Group 2.

Table 3.18: Correlation between metabolic syndrome criteria and diagnostic IR tests at follow-up periods (Group 2) (n=29)

Variable (at baseline)	HOMA-IR		QUICKI	
	r-value (Spearman*)	**p-value**	**r-value** (Spearman*)	**p-value**
Waist circumference	0.04	0.85	-0.04	0.85
Triglycerides	0.32	0.09	-0.32	0.09
HDL-cholesterol	-0.30	0.11	0.30	0.11
Systolic blood pressure	0.25	0.20	-0.25	0.20
Diastolic blood pressure	0.29	0.12	-0.29	0.12
Fasting glucose	**0.56**	**<0.01**	**-0.56**	**<0.01**
Variable (at two months)	**HOMA-IR**		**QUICKI**	
	r-value (Spearman*)	**p-value**	**r-value** (Spearman*)	**p-value**
Waist circumference	0.15	0.43	-0.15	0.43
Triglycerides	0.10	0.62	-0.10	0.62
HDL-cholesterol	-0.33	0.08	0.33	0.08
Systolic blood pressure	0.15	0.43	-0.15	0.43
Diastolic blood pressure	0.14	0.48	-0.14	0.48
Fasting glucose	**0.54**	**<0.01**	**-0.54**	**<0.01**
Variable (at five months)	**HOMA-IR**		**QUICKI**	
	r-value (Spearman*)	**p-value**	**r-value** (Spearman*)	**p-value**
Waist circumference	0.12	0.53	-0.12	0.53
Triglycerides	0.19	0.32	-0.19	0.32
HDL-cholesterol	-0.21	0.27	0.21	-0.27
Systolic blood pressure	0.13	0.51	-0.13	0.51
Diastolic blood pressure	0.00	1.00	0.00	1.00
Fasting glucose**	**0.59**	**<0.01**	**-0.59**	**<0.01**

**Spearman rank correlation co-efficient*

HOMA-IR = homeostasis model assessment-insulin resistance; QUICKI = quantitative insulin sensitivity check index; HDL = high density lipoprotein

Bold and shaded variables indicate statistical significance

The fasting glucose showed a positive correlation (Spearman rank correlation co-efficient) with HOMA-IR and a negative correlation with QUICKI. Therefore, as fasting glucose increased, so did HOMA-IR, whereas the QUICKI decreased. This was observed at all time periods (Table 3.18). This is, however, to be expected given the nature of the mathematical relationship between the two fasting indices.

3.13 DETERMINATION OF HOMA-IR CUT-OFF POINT

3.13.1 General

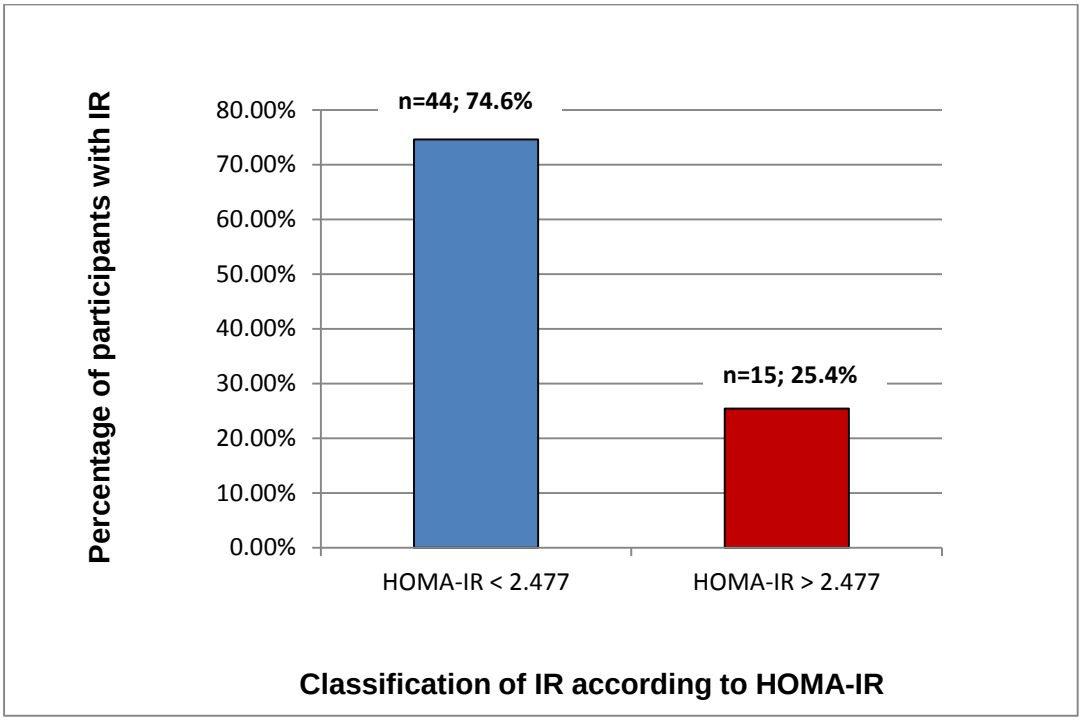
The data of all fifty-nine participants was used to calculate an individualised HOMA-IR value for the study population. Data was categorised into various percentiles and the lower limit of the upper quartile (P75) was chosen as the cut-off point for classifying IR in this study population (Table 3.19). Although there is currently a lack of consensus in terms of a standardised HOMA-IR cut-off point, several studies have adopted the approach of using the lower limit of the upper quartile, namely the 75th percentile, in the calculation of a suitable HOMA-IR value.[293-296]

Table 3.19: Various percentile cut-off points for current study data (n=59)

Percentile classification	Number of participants (n)	Corresponding HOMA-IR value
50th Percentile (P50)	59	1.394
66th Percentile (P66)	59	1.954
75th Percentile (P75)	**59**	**2.477**
80th Percentile (P80)	59	2.766
90th Percentile (P90)	59	3.302

HOMA-IR = homeostasis model assessment-insulin resistance

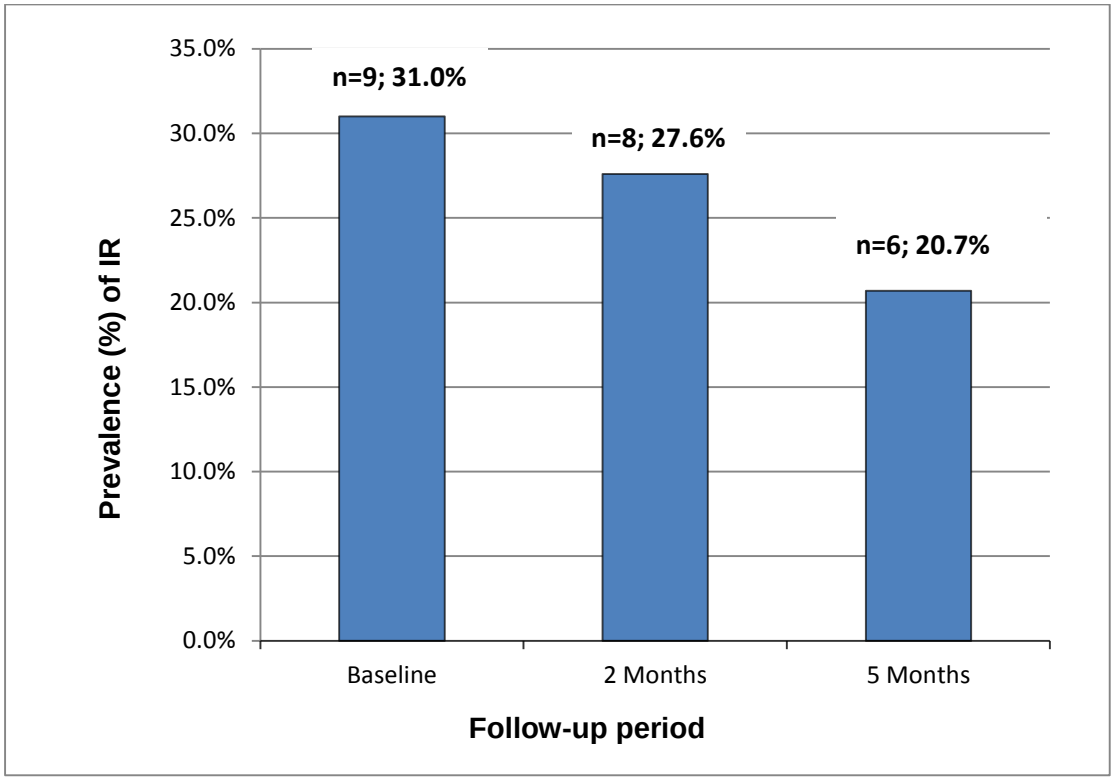
If the above cut-off of HOMA-IR >2.477 is considered to classify a participant with IR, then the following is true for the total study population at baseline: 25.4% of participants (n=15) had HOMA-IR values of >2.477 at baseline, which is shown in Figure 3.22. Of the total number of participants with IR (n=15), males had a total prevalence of 25.0% (n=12/48) and females, 27.3% (n=3/11).



IR = insulin resistance; HOMA-IR = homeostasis model assessment-insulin resistance

Figure 3.22: Classification of total population at baseline according to determined HOMA-IR cut-off point (n=59)

Of the follow-up participants (n=29), there was an IR prevalence rate of 31.0% at baseline (n=9), 27.6% at two months (n=8) and 20.7% at five months (n=6). This was determined using the same HOMA-IR cut-off point determined above (2.477) and is depicted in Figure 3.23.



IR = insulin resistance; HOMA-IR = homeostasis model assessment-insulin resistance

Figure 3.23: Prevalence of IR according to HOMA-IR values in Group 2 (n=29)

Of the nine participants that were classified as having IR in Group 2 at baseline, four of these persons were still classified as having IR at the five-month follow-up period (n=4; 44.4%), whereas two participants (n=2) who presented with normal HOMA-IR levels at baseline developed IR at both two and five-month follow-up periods.

This data is presented slightly differently in Table 3.20 and shows how the IR participants were distributed over the follow-up period. Some patients who did not have IR at baseline developed it at a later stage during the follow-up.

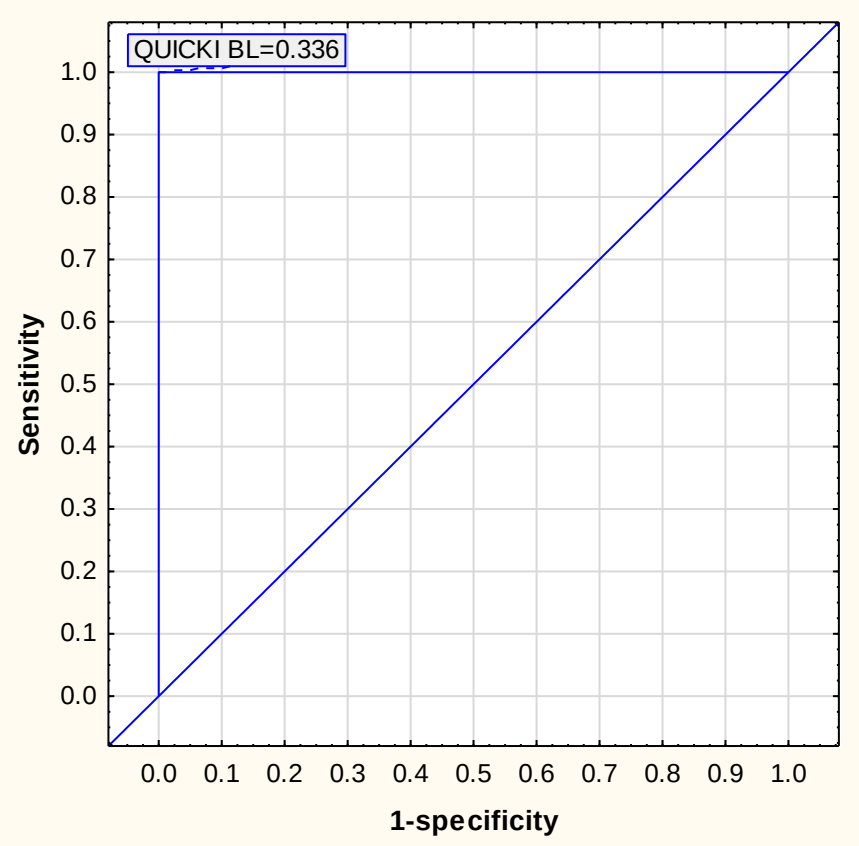
Table 3.20: Changes in IR participants over all three time periods (baseline, two and five months)

Time period	Number of participants classified with IR (n)
IR at baseline only	3
IR at baseline and two months	2
IR at baseline, two and five months	2
IR at two months only	2
IR at two and five months	2
IR at baseline and five months	2
IR at five months	0

IR = insulin resistance

3.13.2 Calculation of Corresponding QUICKI Cut-off Point

A ROC analysis was done to determine the optimal QUICKI cut-off point at baseline. This analysis was based on the pre-determined HOMA-IR cut-off value of 2.477 (P75) to identify IR in study participants. This test, therefore, assumes that the HOMA-IR value is the true reading. Upon performing this statistical analysis, a corresponding QUICKI value of 0.336 was identified using the Youden index (Figure 3.24). In contrast to the HOMA-IR value (where the higher the value, the more insulin resistant an individual is), the QUICKI is the opposite as the lower the value, the more IR is present.



QUICKI = quantitative insulin sensitivity check index

Figure 3.24: Scatterplot of sensitivity against 1-specificity

As the QUICKI and HOMA-IR are mathematically related, the identified QUICKI cut-off mentioned above classified the same participants as having IR as the HOMA-IR cut-off value (i.e. 15 of the 59 participants at baseline). The sensitivity and specificity of the two cut-off values was, therefore, 100%. This confirms the accuracy between the two measures.

3.13.3 Exclusion of Participants Identified Post-study Entry with Metabolic Syndrome

As was mentioned previously in Chapter 3.11, it was discovered that two of the female participants entered onto the study were identified as having metabolic syndrome according to the IDF guidelines, whereas the ATP III guidelines only identified one of these two participants as having the syndrome at baseline. It was decided to recalculate the HOMA-IR cut-off point/s by excluding both the IDF and ATP III participants from a sub-analysis in order to improve accuracy of the results, as the syndrome is known

to be synonymous with IR prevalence. The reworked cut-off points were still calculated by using the lower limit of the upper quartile and are shown in Table 3.21.

In addition to this, the two participants who had raised fasting glucose levels (although not confirmed diabetics) were also excluded in a sub-analysis to determine the effect on the HOMA-IR cut-off value. This sub-analysis produced the same HOMA-IR cut-off as the IDF excluded participants (seen below in Table 3.21)

Table 3.21: Recalculated HOMA-IR cut-off point with exclusion of two participants

Percentile classification	Number of participants (n)	Corresponding HOMA-IR value	Baseline IR prevalence
75th Percentile (P75)	59	2.477	25.4% (n=15)
75th Percentile (P75)	57 *(Excluding n=2: IDF criteria)*	**2.334**	**24.6% (n=14)**
75th Percentile (P75)	58 *(Excluding n=1: ATP III criteria)*	**2.276**	**25.9% (n=15)**
75th Percentile (P75)	57 *(Excluding n=2: possible DM)*	**2.334**	**24.6% (n=14)**

HOMA-IR = homeostasis model assessment-insulin resistance; IR = insulin resistance; DM = diabetes mellitus; IDF = International Diabetes Federation; ATP = Adult Treatment Panel

If comparing the re-calculated results to those of the total study population (n=59), the prevalence rates of IR of the total population was 25.4%, whereas the prevalence in the IDF re-calculated group was 24.6% (n=14) and 25.9% (n=15) in the ATP-III re-calculated group. The 'IDF' group displays a 0.8% lower IR prevalence when compared with the total study population, whereas the 'ATP III' group shows a 0.5% increase in the prevalence of IR at baseline.

When considering the impact of the new HOMA-IR cut-off points on the prevalence rates of Group 2 (n=29), the differences are shown in Table 3.22.

Table 3.22: Comparison of different HOMA-IR values in Group 2 (n=29)

	Initial HOMA-IR cut-off (2.477)		Re-calculated 'IDF' HOMA-IR cut-off (2.334)		Re-calculated 'ATP III' HOMA-IR cut-off (2.276)	
	n	**%**	**n**	**%**	**n**	**%**
Baseline	9	31.0%	9	31.0%	**10**	**34.5%**
2 Months	8	27.6%	**11**	**37.9%**	**12**	**41.4%**
5 Months	6	20.7%	6	20.7%	6	20.7%

HOMA-IR = homeostasis model assessment-insulin resistance; IDF = International Diabetes Federation; ATP = Adult Treatment Panel

Therefore, despite slight discrepancies among the number of participants being classified as having IR at baseline and 2 months using the three different HOMA-IR cut-off points, the five-month result shows that there was no difference in classification of participants as having IR between initial HOMA-IR cut-off of 2.477 and the re-calculated values (n=6, 20.7%).

3.13.4 Regression Analysis to Investigate Role of Different Variables on Insulin Resistance

A full multiple regression test was done. Thereafter, the best predictor variables (for both anthropometry and biochemistry data sets) were determined with an all subsets multiple regression analysis using Mallow's Cp Criterium for selection of the best predictor variables.

The full multiple regression model for anthropometrical data yielded the following output which explained only 22.5% of the variation in IR (HOMA-IR at baseline). The all subsets approach with Mallow's Cp Criterion selection, yielded the following anthropometrical variables [waist circumference (r=0.507; p=0.011), sum of skinfold's (r=0.353; p=0.039) and fat mass (r=-2.419; p=0.08)] as the best predictors of IR (HOMA-IR at baseline). This model explained only 20.4% of the variation in IR, showing that IR can mostly be explained with the above-mentioned anthropometrical variables. The other variables do not contribute to IR at all.

The full multiple regression model for the biochemical data yields the following output which explained only 99.0% of the variation in IR (HOMA-IR at baseline). The all subsets approach with Mallow's Cp Criterion selection yielded the fasting glucose (r=0.524; p=0.000) and fasting insulin (r=0.291; p=0.000) as the best predictors of IR. This model explains 98.9% of the variation in HOMA-IR levels (and ultimately IR). Thus IR is mostly associated with fasting glucose and fasting insulin, whereas other variables contribute minutely, if at all.

The results of the multiple regression therefore show that fasting glucose and fasting insulin measurements are the best predictors of IR, whereas there are almost no anthropometrical variables that are significantly associated with IR development. The biochemical results are logical as both fasting glucose and fasting insulin are individual components of the formula to calculate HOMA-IR.

3.14 INSULIN RESISTANT GROUP vs. NON-INSULIN RESISTANT GROUP

3.14.1 Demographic Data

This group consisted of fifteen participants (n=15), who were identified after data collection and analysis occurred. Of these IR participants, twelve were male (80.0%) and the remaining three were female (20.0%). Nine of the IR group were black African (60.0%), whereas five were coloured (33.3%) and the remaining participant was white (n=1; 6.7%).

As was noted with the demographic data, the statistical validity of comparing the IR and non-IR groups has been accounted for with appropriate statistical tests (Mean Square Error).

3.14.2 Categorical Variables

When considering Table 3.23 below, the only significant differences found among the categorical variables when comparing the IR with the non-IR group were found in the interpretations of the percentage body fat, subscapular skinfold and sum of subscapular and triceps skinfold (p-values indicated in bold in Table 3.23).

Table 3.23: Comparison of baseline variables of IR group vs. non-IR group (categorical variables) (n=59)

Variable	Groups assessed	p-value **
Gender	IR vs. non-IR	0.877
Age Groups*	IR vs. non-IR	0.210
Race	IR vs. non-IR	0.816
BMI classification	IR vs. non-IR	0.217
Waist circumference classification	IR vs. non-IR	0.122
Waist:hip ratio classification	IR vs. non-IR	0.831
Frame size	IR vs. non-IR	0.193
Interpretation of percentage body fat	**IR vs. non-IR**	**0.022**
Subscapular skinfold interpretation	**IR vs. non-IR**	**0.021**
Triceps skinfold interpretation	IR vs. non-IR	0.080
Interpretation of sum of subscapular and triceps skinfolds	**IR vs. non-IR**	**0.042**
HDL-cholesterol classification	IR vs. non-IR	0.293

**Age groups concerned: 18-30 years; 31-45 years; 46-65 years*

***M-L Chi-square test = Maximum-likelihood chi square test*

BMI = body mass index; HDL = high density lipoprotein

Bold and shaded variables indicate statistical significance

With regard to the interpretation of percentage body fat between the two groups, the ‘underweight’ category showed the largest difference (non-IR: n=18, 40.9% vs. IR: n=2, 13.3%). This was corroborated as the IR group showed a higher percentage body fat (although not significant) when compared with their non-IR counterparts [as the former had participants classified in the ‘heavy’ and ‘excessive’ categories (n=2, 13.3% and n=1, 6.7%) respectively].

The subscapular skinfold interpretation also showed a difference between the IR and non-IR groups, as the ‘below average’ category had more representation from the non-IR group (n=16, 36.4%) compared with the IR group (n=2, 13.3%). Conversely, the ‘average’ category had more participants from the IR group (n=6, 40.0%) vs. that non-IR group (n=4, 9.1%). This distribution is clarified as the mean skinfold values were also higher among the IR group, although not significantly so.

The sum of triceps and subscapular skinfolds showed similar results, in that the largest differences were seen in those participants classified in the ‘lean’ category (non-IR: n=24, 54.6% vs. IR: n=4, 26.7%) where the non-IR group had the majority. The ‘average’ category showed the converse, with the non-IR group showing minority representation when compared with the IR group (non-IR: n= 8, 18.2% vs. IR: n=7 (46.7%). Once again, this is confirmed as the IR group had a higher mean value, although it was also not significant.

3.14.3 Continuous Variables

When considering the differences shown in Table 3.24 between the IR and non-IR group with regard to the biochemical values, only fasting insulin and the diagnostic IR tests showed significant differences. The latter is due to the fact that fasting insulin is a component of the various calculations used in the diagnostic IR tests. Although diastolic blood pressure was very close to the significant level cut-off point, no difference could be detected, although it could perhaps be interpreted as showing a trend towards significance.

Table 3.24: Comparison of baseline variables of IR group vs. non-IR group (continuous variables) (n=59)

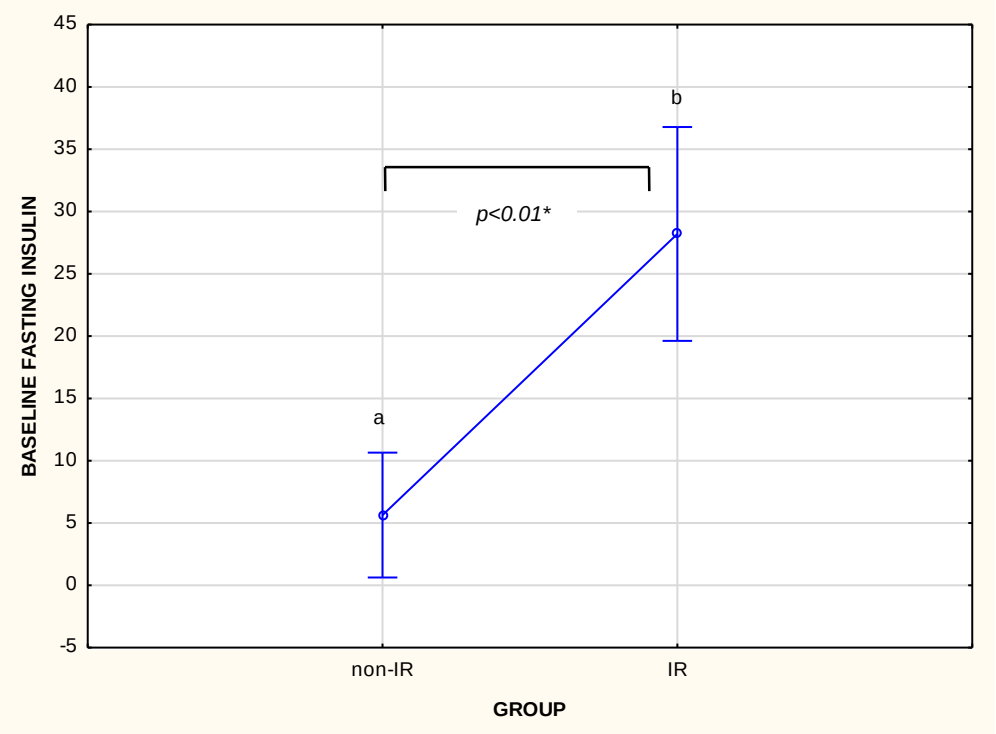
Variable (Baseline)	Unit	non-IR Group (n=44) Mean (SD)	IR Group (n=15) Mean (SD)	p-value *
Age	**years**	**35.43 (12.02)**	**29.60 (11.29)**	**0.04**
Systolic blood pressure	mmHg	119.23 (17.78)	125.13 (11.57)	0.13
Diastolic blood pressure	mmHg	75.45 (12.46)	79.93 (10.42)	0.05
Albumin	g/L	38.75 (4.58)	41.00 (3.16)	0.17
Fasting glucose	mmol/L	4.71 (0.69)	5.13 (1.02)	0.16
CRP	mmol/L	64.10 (53.82)	48.91 (41.01)	0.51
Fasting insulin	**mU/l**	**5.63 (2.43)**	**28.20 (33.21)**	**<0.01**
Total cholesterol	mg/L	3.39 (0.87)	3.71 (0.95)	0.30
Triglycerides	mmol/L	0.88 (0.35)	0.95 (0.27)	0.38
HDL-cholesterol	mmol/L	0.99 (0.33)	0.93 (0.26)	0.56
LDL-cholesterol	mmol/L	1.98 (0.65)	2.35 (0.80)	0.24
White cell count	10^9/l	8.79 (3.68)	8.99 (3.30)	0.68
HOMA-IR	**-**	**1.18 (0.54)**	**7.22 (10.32)**	**<0.01**
QUICKI	**-**	**0.39 (0.04)**	**0.31 (0.03)**	**<0.01**

** Mann-Whitney U test*

SD = standard deviation; CRP = C-reactive protein; HDL = high density lipoprotein; LDL = low density lipoprotein; HOMA-IR = homeostasis model assessment-insulin resistance; QUICKI = quantitative insulin sensitivity check index

Bold and shaded variables indicate statistical significance

A graphic representation of the significant difference seen between the mean fasting insulin value of the IR group vs. that of the non-IR group is shown in Figure 3.25.

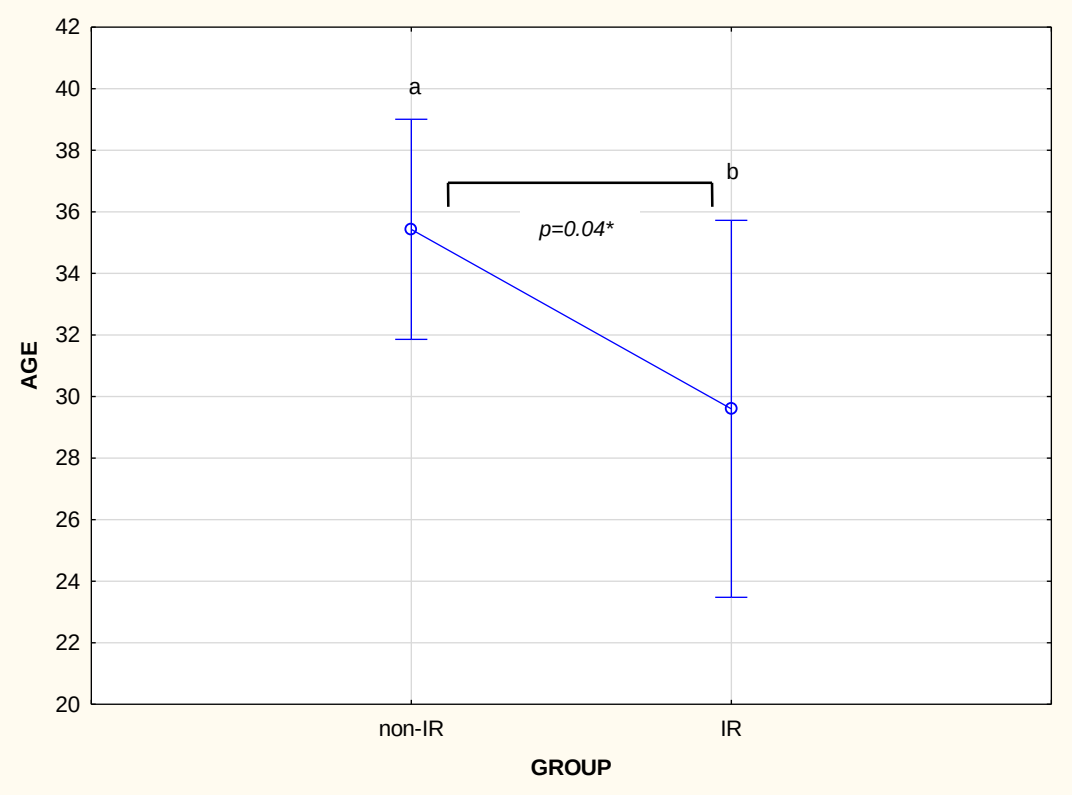


IR = insulin resistance

** Mann-Whitney U test*

Figure 3.25: Comparison between IR and non-IR group vs. fasting insulin levels of participants (n=59)

With regard to demographics, only age displayed a significant difference between the IR and non-IR groups. Figure 3.26 shows that the study participants in the IR groups were significantly younger than their counterparts (p=0.04; Mann-Whitney U test).



IR = insulin resistance

** Mann-Whitney U test*

Figure 3.26: Comparison between IR and non-IR group vs. age of participants (n=59)

3.15 BASELINE SPUTUM RESULTS

3.15.1 General

Of the total study population (n=59), only fifty-six participants (94.9%) had results available at baseline. Fifty-two participants in the total study population of fifty-nine (88.14%) were found to have tested positive via the Gene Xpert test, with all of these participants being drug sensitive (rifampicin susceptible). Individualised sputum test results (according to smear microscopy discussed in Chapter 1.2.6) of the participants at baseline are shown in Table 3.25 below.

Table 3.25: Sputum results of baseline participants (n=56)

Sputum results	Total study population (n=56) n (%)	Group 1 (n=29) n (%)	Group 2 (n=27) n (%)
Smear-negative	14 (25.0%)	**9 (31.0%)**	5 (18.5%)
Scanty-positive	8 (14.3%)	4 (13.8%)	4 (14.8%)
Smear-positive (+)	9 (16.0%)	5 (17.2%)	4 (18.1%)
Smear-positive (++)	8 (14.3%)	5 (17.2%)	3 (11.1%)
Smear-positive (+++)	**17 (30.4%)**	6 (20.7%)	**11 (40.7%)**

The largest group of patients fell into the 'smear-positive (+++)' category when considering the baseline results (n=17; 30.4%), although this was closely followed by the 'smear-negative' group (n=14; 25.0%). Group 1 had the majority of participants fall into the 'smear-negative' group (n=9; 31.0%) whilst Group 2 saw most of their participants classified in the 'smear-positive (+++)' group (n=11; 40.7%). The majority values are indicated in Table 3.24 above in bold and are shaded.

In order to simplify the comparison between the various sputum groups, results were classified as either 'smear-negative', 'indecisive' or 'smear-positive'. A participant was classified as having an 'indecisive' sputum test if the test needed to be repeated due to uncertainty of the initial results, which is often what was found if the result came back as 'scanty positive'. In clinical practice, a 'scanty' result is often viewed as being a negative test and is thus repeated for good measure.

The distribution between the three sputum categories is highlighted in Figure 3.27.

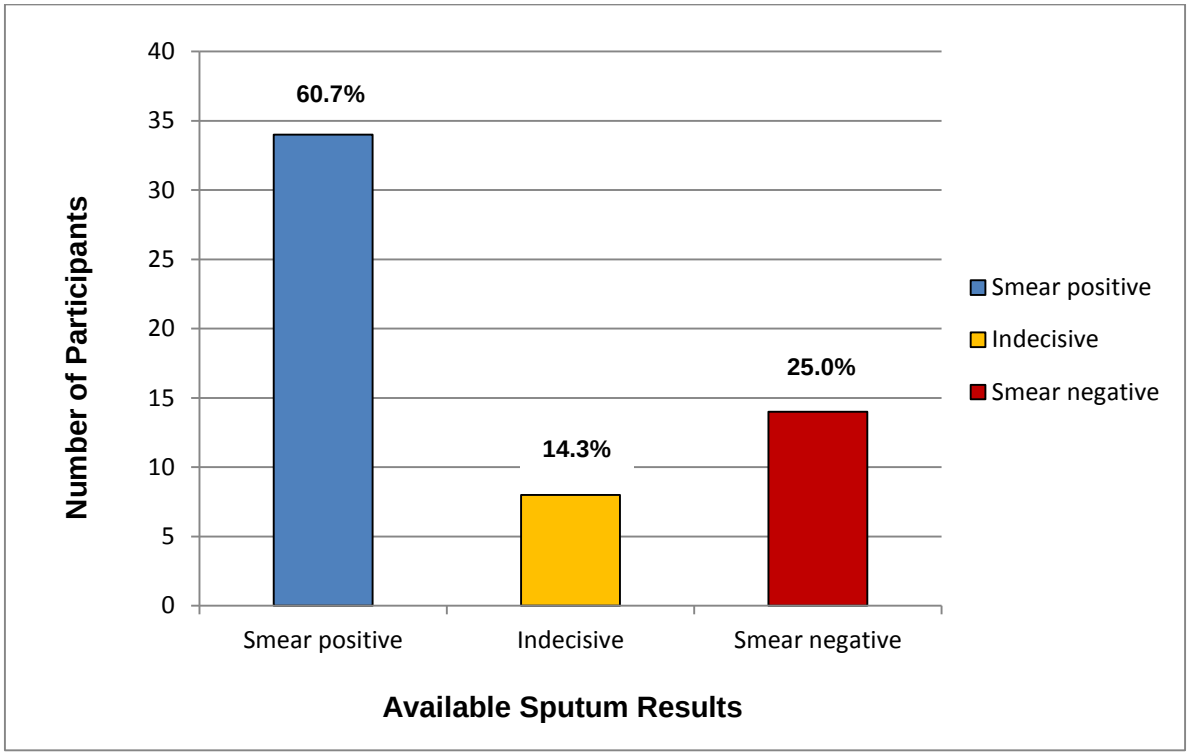


Figure 3.27: Available sputum results of study population at baseline (n=56)

If the participants in the 'indecisive' and 'smear-negative' groups were added together, twenty-two participants would fall into this 'new' smear-negative group (n=22, 39.3%), whilst the remaining 60.7% (n=34) would be classified as 'smear-positive'.

3.15.2 Categorical Variables (Anthropometry and Demographics)

Comparisons were made between the smear-positive and-negative groups to ascertain whether any differences were present for any of the categorical anthropometrical variables assessed. The results are tabulated in Table 3.26 below and show that no significant differences, apart from 'age group' were found between any of the variables. The 'age group' variable showed a significant difference between smear-positive and-negative groups in the 18 – 30 year old age group, with more patients in the 'smear-positive' group.

Table 3.26: Comparison of baseline categorical variables of smear-positive vs. smear-negative groups (categorical variables) (n=56)

Variable	Groups assessed	p-value **
Gender	Smear-negative vs. positive	0.354
Age groups*	**Smear-negative vs. positive**	**0.039**
Race	Smear-negative vs. positive	0.368
BMI classification	Smear-negative vs. positive	0.155
Waist circumference classification	Smear-negative vs. positive	0.827
Waist:hip ratio classification	Smear-negative vs. positive	0.158
Frame size	Smear-negative vs. positive	0.813
Interpretation of percentage body fat	Smear-negative vs. positive	0.111
Subscapular skinfold interpretation	Smear-negative vs. positive	0.985
Triceps skinfold interpretation	Smear-negative vs. positive	0.366
Interpretation of sum of subscapular and triceps skinfolds	Smear-negative vs. positive	0.544
HDL-cholesterol classification	Smear-negative vs. positive	0.588

**Age groups concerned: 18-30 years; 31-45 years; 46-65 years*

***M-L Chi-square test = Maximum-likelihood chi square test*

BMI = body mass index

Bold and shaded variables indicate statistical significance

3.15.3 Biochemistry, Blood Pressure and Diagnostic Insulin Resistance Tests

Table 3.27 indicates the comparison between the smear-negative and positive groups in terms of any differences occurring with regard to biochemical variables (bold and shaded variables indicate statistical significance).

Table 3.27: Comparison of baseline biochemical, blood pressure and diagnostic IR variables of smear-positive vs. smear-negative groups (n=56)

Variable (Baseline)	Unit	Smear-negative (n=22) Mean (SD)	Smear-positive (n=34) Mean (SD)	p-value *
Systolic blood pressure	mmHg	120.32 (15.56)	121.59 (17.70)	0.97
Diastolic blood pressure	mmHg	76.63 (12.78)	76.47 (11.85)	0.89
Albumin	g/L	39.59 (4.22)	38.88 (4.41)	0.41
Fasting glucose	**mmol/L**	**5.08 (1.00)**	**4.58 (0.52)**	**0.04**
CRP	mmol/L	50.09 (52.34)	67.80 (50.65)	0.12
Fasting insulin	mU/l	10.03 (11.03)	8.54 (1.46)	0.70
Total cholesterol	mg/L	3.46 (0.77)	3.45 (0.98)	0.79
Triglycerides	mmol/L	0.83 (0.27)	0.94 (0.37)	0.37
HDL-cholesterol	mmol/L	1.03 (0.32)	0.95 (0.33)	0.31
LDL-cholesterol	mmol/L	2.04 (0.52)	2.07 (0.79)	0.91
White cell count	10^9/l	8.57 (3.75)	8.95 (3.49)	0.62

Variable (Baseline)	Unit	Smear-negative (n=22) Mean (SD)	Smear-positive (n=34) Mean (SD)	p-value *
HOMA-IR	-	2.42 (3.34)	1.78 (1.46)	0.96
QUICKI	-	0.36 (0.05)	0.37 (0.05)	0.96

** Mann-Whitney U test; SD = standard deviation; CRP = C-reactive protein; HDL = high density lipoprotein; LDL = low density lipoprotein; HOMA-IR = homeostasis model assessment-insulin resistance; QUICKI = quantitative insulin sensitivity check index*

The fasting glucose of the participants with sputum-negative results were significantly higher than those of their smear-positive counterparts (p=0.04; Mann-Whitney U-test)

3.15.4 Baseline Sputum Results (between IR and non-IR groups)

Of the original group of fifteen participants identified as having IR (Chapter 3.14), only 14 of these had available sputum results (93.3%). The differences between the IR and non-IR groups in terms of sputum results are shown in Table 3.28.

Table 3.28: Sputum results of baseline participants according to IR vs. non-IR groups (n=56)

Sputum results	Total study population (n=56) n (%)	IR Group (n=14) n (%)	non-IR Group (n=42) n (%)
Smear-negative	14 (25.0%)	4 (28.6%)	10 (23.8%)
Scanty-positive	8 (14.3%)	1 (7.1%)	7 (16.7%)
Smear-positive (+)	9 (16.1%)	2 (14.3%)	7 (16.7%)
Smear-positive (++)	8 (14.3%)	2 (14.3%)	6 (14.3%)
Smear-positive (+++)	**17 (30.4%)**	**5 (35.7%)**	**12 (28.6%)**
TOTAL	**56**	**14**	**42**

IR = insulin resistant

For both the IR Group and non-IR group, most participants fell into the 'smear-positive (+++)' group, although this was not the majority of participants. There were no significant differences between the IR and non-IR groups when compared with one another (p=0.899; M-L Chi-square).

3.16 FOLLOW-UP SPUTUM RESULTS

3.16.1 General

Of the participants in Group 2 with available sputum results at baseline (n=27), there were twenty-three participants who had available sputum results at two months (n=23, 85.2%) and nineteen participants at five months (n=19, 70.4%) (Table 3.29).

Table 3.29: Available sputum results of Group 2 over time

Sputum result classification	Baseline (n=27)	Two months (n=23)	Five months (n=19)
Smear-negative	n=5 (18.5%)	n=19 (82.6%)	n=18 (94.7%)
Indecisive	n=4 (14.8%)	n=2 (8.7%)	n=1 (5.3%)
Smear-positive	n=18 (66.7%)	n=2 (8.7%)	-

Participant numbers seem to drop quite rapidly from baseline sputum results to the follow-ups between months two and five. As only the participants in Group 2 had sputum values recorded at two and five months, this accounted for the difference.

Only one (n=1) of the smear-negative patients was re-tested at two months and found to be negative. All of the other baseline smear-negative patients in Group 2 were not tested beyond the baseline measurement. Of the four indecisive smear patients at baseline, all had converted to smear-negative by two months (100.0%). Upon consideration of the baseline smear-positive individuals in Group 2 (n=18), fourteen of these (n=14, 77.8%) had converted by two months, whilst two (n=2, 11.1%) patients had converted to an indecisive result and two patients remained smear-positive (n=2, 11.1%).

Only one (n=1) of the smear-indecisive patients was re-tested at five months and this was also a negative result. The smear-positive patients who had converted at two months (n=14) also tested negative at five month follow-up. Both of the patients with positive smears at two months had converted by five months (n=2), while one of the patients with an indecisive smear showed a negative sputum result (n=1). The remaining participant (n=1) from the two-month indecisive group still had an indecisive result at five months.

Therefore, of the original eighteen participants who presented with positive smears at baseline, 94.4% (n=17) had converted by the five-month follow-up period. Table 3.30 indicates any differences in overall sputum results over time.

Table 3.30: Sputum results over follow-up period

Time period	p-value	Test	Outcome
Baseline sputum → two-month sputum	**p<0.001**	***McNemar-Bowker***	**Reject null hypothesis**
Baseline sputum → five-month sputum	**p<0.001**	***McNemar-Bowker***	**Reject null hypothesis**
Two month sputum → five-month sputum	p=0.317	*McNemar*	Do not reject null hypothesis

Bold and shaded variables indicate statistical significance

There was, therefore, a significant difference between sputum results between baseline and two months (p<0.001), as well as baseline and five months (p<0.001) which was confirmed via a McNemar-Bowker test (a variation of the chi-squared test). The absence of a difference between two-month and five-month sputum results was shown via a McNemar test.

The changes between sputum results during the follow-up period as compared with HOMA-IR and QUICKI values are shown in Table 3.31.

Table 3.31: Changes in sputum results over time compared with HOMA-IR and QUICKI values

Variable	ANOVA (F-test)	Smear-negative Mean (SD)	Indecisive Mean (SD)	Smear-positive Mean (SD)	p-value*
HOMA-IR					
Baseline	F (2, 53) = 1.70	3.01 (4.08)	1.40 (0.80)	1.78 (1.46)	0.76
Two months	F (2, 21) = 0.37	3.41 (5.12)	1.31 (0.23)	0.93 (0.44)	0.47
Five months	F (1, 18) = 0.14	2.03 (1.21)	1.56 (-)	-	1.00
QUICKI					
Baseline	F (2, 53) = 0.26	0.36 (0.06)	0.37 (0.03)	0.37 (0.05)	0.76
Two months	F (2, 21) = 0.35	0.36 (0.06)	0.37 (0.01)	0.39 (0.03)	0.47
Five months	F (1, 18) = 0.02	0.35 (0.03)	0.36 (-)	-	1.00

**Kruskal-Wallis*

SD = standard deviation; HOMA-IR = homeostasis model assessment-insulin resistance; QUICKI = quantitative insulin sensitivity check index

As can be seen in Table 3.31, it appears as if the smear-negative group have both higher HOMA-IR and lower QUICKI values at all periods during the study, although none showed significant differences.

3.16.2 Follow-up Sputum Results (between IR and non-IR groups)

Table 3.32 illustrates the changes the baseline IR group experienced over time. There were only nine of the original fifteen participants in Group 2 (follow-up group) and only eight of these had sputum results available at baseline.

Table 3.32: Comparison between Group 2 IR and non-IR group at two and five-month follow-up periods

Sputum result classification	IR Group	non-IR Group
Baseline	**n=8**	**n=19**
Smear-negative	n=1 (12.5%)	n=4 (21.1%)
Indecisive	n=1 (12.5%)	n=3 (15.8%)
Smear-positive	n=6 (75.0%)	n=12 (63.2%)
Two month follow-up	**n=7**	**n=16**
Smear-negative	n=7 (100.0%)	n=12 (75.0%)
Indecisive	n=0 (0.0%)	n=2 (12.5%)
Smear-positive	n=0 (0.0%)	n=2 (12.5%)
Five month follow-up	**n=7**	**n=12**
Smear-negative	n=7 (100.0%)	n=11 (91.7%)
Indecisive	n=0 (0.0%)	n=1 (8.3%)
Smear-positive	n=0 (0.0%)	n=0 (0.0%)

IR = insulin resistant

As can be seen by the data presented in Table 3.32, all of the IR participants presented with negative smears at both two- and five-months, barring one participant who was not re-tested. All six of the smear-positive patients smear-converted by two months (n=6; 100.0%).

Despite the IR group having all their scanty-positive and smear-positive participants convert to smear-negative by the two month follow-up, no differences between the IR and non-IR groups were shown when comparing sputum results at two months (p=0.330; M-L Chi-square) and five months (p=0.304; M-L Chi-square).

3.17 SUMMARY OF MAIN FINDINGS

Table 3.33 provides a tabulated summary of the main study findings discussed in this chapter.

Table 3.33: Summary of main study findings

OUTCOME CATEGORY	MAIN FINDINGS
Anthropometry	• The majority (61.0%) of participants in the study had a normal BMI although some were classified as overweight (BMI>25 kg/m^2). • Despite the majority of patients having a BMI within the normal range, the total body fat was still relatively low, and most patients were classified as being either 'underweight' or 'slim' when assessed. • The majority of anthropometrical measurements showed a significant increase whilst the patients were on treatment, with the greatest changes taking place during the intensive phase (first two months of treatment). • Some patients failed to gain more than 5% weight over the five-month period and/or remained in the underweight BMI category. • There was a significant increase in both fat and fat free mass over time. • The increase in fat mass seemed to be associated with gains in both the peripheral and trunk area (albeit slightly more in the trunk area).
Biochemistry	• The most noticeable derangements in baseline biochemical values showed the majority of patients having an increased CRP level and a decreased HDL-cholesterol level • There were relatively few presentations of hyperglycaemia and/or abnormal insulin levels in the study although IR rates were found to be high. • CRP, albumin and white cell count all showed an improvement over time (especially in the intensive treatment phase). • Although the cholesterol levels (total, LDL and HDL) increased in the first two months of treatment, indicating a possible improved response to infection, there were no differences noted over the last few months.
Diagnostic IR tests	• Using the calculated cut-off point for HOMA-IR (2.477), the IR prevalence at baseline was 25.4%, which equates to 1 in 4 persons with newly diagnosed PTB having IR. • The HOMA-IR and QUICKI levels also improved with time, although not significantly, which may indicate a seeming reduction in IR. • Some patients who had IR at baseline presented with normal levels at two months but then redeveloped IR at five months. In addition, some patients were non-IR at baseline, but developed it at two and five months.
Metabolic Syndrome	• The metabolic syndrome criteria used (IDF and ATP III) failed to classify the majority of patients who presented with IR according to HOMA-IR and QUICKI calculated cut-offs. • Adjusting for patients with the metabolic syndrome according to both IDF and ATP III criteria had little effect on the total IR prevalence at baseline.
IR vs. Non-IR participants	• Differences noted in IR patients compared with their non-IR counterparts: IR patients were younger, had a significantly higher interpretation of skinfolds and percentage body fat and higher fasting insulin values. • Although no other significant results were noted, IR patients generally showed a pattern with markers of the metabolic syndrome (increased fasting glucose, triglycerides and blood pressure). • Mean CRP levels were lower in patients with IR compared with their non-IR counterparts although not significantly so.
Sputum results	• The majority of patients converted to sputum smear-negative by the five-month mark, signifying an improved clinical state. • When comparing differences between the sputum smear-positive and negative groups, only the fasting glucose measurement was significant; the sputum-positive group had a lower fasting glucose level than the negative group and ultimately 'less' IR according to HOMA-IR and QUICKI.

3.18 HYPOTHESIS TESTING

According to the union-intersection principle,[299,300] which states that for a null hypothesis to be true, it needs to be true for all variables, the following can be deduced (see individual p-values in text):

- The hypothesis stating that there is no difference in **anthropometrics or body composition** with PTB at baseline and follow-up periods was **rejected**.
- The hypothesis stating that there is no difference in **biochemistry** with PTB at baseline and follow-up periods was **rejected**.
- The hypothesis stating that there is no difference in **blood pressure** with PTB at baseline and follow-up periods was **not rejected**.
- The hypothesis stating that there is no difference in **IR status** (via HOMA-IR/QUICKI) with PTB at baseline and follow-up periods was **not rejected**.
- The hypothesis stating that there is no difference in **sputum TB results** with PTB at baseline and follow-up periods was **rejected.**
- The hypothesis stating that there is no difference in anthropometrics, body composition, blood pressure or sputum results **between IR and non-IR** adults with PTB at baseline was **rejected**.
- The hypothesis stating that there is no difference in anthropometrics, body composition, blood pressure or sputum results **between smear-negative and smear-positive** adults with PTB at baseline was **rejected.**

CHAPTER 4: DISCUSSION

Given the current worldwide status of tuberculosis as the second leading cause of mortality attributed to an infectious disease, it is evident that this condition is a large-scale threat to achieving the Millennium Development Goals, more recently the Sustainable Development Goals, in the years to come. South Africa, given its high prevalence of both communicable and non-communicable diseases, has unfortunately not escaped its impact or undesirable consequences. Exploration of the bi-directional relationship between tuberculosis and diabetes has been gaining recognition in recent years, albeit more from a 'diabetes resulting in tuberculosis' angle.

Insulin resistance is a common occurrence in a multitude of conditions, of which diabetes and metabolic syndrome are but a few.[14,15,150,151] Insulin resistance unfortunately has far-reaching and detrimental health outcomes for susceptible individuals, specifically relating to cardiovascular and diabetes-related incidents.[9] Given the recent description in the literature of impaired glucose tolerance, distortions in carbohydrate metabolism and altered insulin action among newly diagnosed TB patients[257-259] it was considered prudent to investigate if there was an association between TB and the development of IR in newly diagnosed adults with pulmonary tuberculosis (PTB) in the Western Sub-District of the Cape Metropole region of South Africa.

4.1 STUDY POPULATION

Of the total number of TB patients admitted to the recruitment site during the period of data collection, 313 individuals met the criteria for further screening by way of appropriate age, being newly registered individuals, as well as presenting with PTB. These participants were further whittled down to those who presented with an HIV-negative status. This equated to the majority of screened participants in this particular group being HIV-positive (61.3%), which is in line with current statistics reporting a 62.0% prevalence rate of HIV-positive TB patients in South Africa.[18] Statistics from Albow Gardens clinic (2014) tend to support the national findings, with an HIV-positive rate of 52.5% among all TB patients.[36] Although these prevalence rates differ slightly, they confirm the overwhelming burden of the HIV/TB pandemic currently seen in Sub-Saharan Africa.

Of the remaining HIV-negative persons, 20.7% were not contactable (n=17) or did not honour the initial appointment with the researcher (n=8). If one considers that approximately one-third of patients do not complete their recommended anti-tuberculosis treatment, this rate of non-contactable or defaulting patients is to be expected.[58]

Of the potential participants, seven were classified as obese (having a BMI ≥30 kg/m^2), which equates to a 5.8% prevalence rate. Despite this figure being lower than the national obesity prevalence among females (39.2%) and males (10.6%) in the general population,[301] this is of concern because although TB patients are often viewed as being malnourished, undernutrition is more often than not the norm.[50,53] The presence of overweight/obesity in this population could be symbolic of the double burden of disease often seen in developing countries such as South Africa. Obesity is also often considered to be a slight protective factor against TB disease.[271]

Estimates published in 2012 by the Society for Endocrinology, Metabolism and Diabetes in South Africa (SEMSDA), regarding diabetes prevalence in South Africa, show figures of approximately 6.5% in persons aged 20 - 79 years, although this could be as high as 13% in urban populations.[3] In 2013, the South African National Health and Nutrition Examination Survey (SANHANES-1) diagnosed diabetes in 9.5% of participants, which is in agreement with the above SEMSDA data.[301] Only 3.3% (n=4) of the potential candidates in this study (with PTB, HIV-negative and newly registered) had confirmed diabetes before commencing with TB treatment. Although in the lower range, this prevalence rate is in agreement with findings by Jeon et al. in 2010 that showed the TB prevalence rate of current diabetic patients to be between 1.7% and 36%.[251] The prevalence rate of diagnosed DM in this particular study was, however, noticeably lower when compared with other recent studies in both developing and developed countries, ranging between 14.8% and 44% (albeit with a range of diagnostic tools).[254,261-264] It is important to bear in mind that the latter studies included patients who were diagnosed with DM after beginning treatment for TB, whilst the prevalence rate mentioned here refers to participants diagnosed prior to treatment commencement and thus excluded from participation. Since the 3.3% of participants with confirmed diabetes were newly registered PTB patients and were HIV-negative, this could have played a role in the lower prevalence rate of diabetes among TB patients. A study conducted in Africa documented a two-fold increase in the prevalence of diabetes in TB vs. non-TB patients,[302] which is considerably higher than that of the current study population. The low rates of diagnosed diabetes found in the current study could also be attributed to inadequate DM screening procedures by health care staff during TB testing. Assessing patients for both TB and DM is a growing need in combating the problem at hand.[38] Participants who were excluded on the basis of obesity could also have presented with DM, although this was not verified.

The present study population showed a 13.6% loss to follow-up. This relatively low rate could be advantageous, especially since the majority of the data from these defaulting participants could still be used in the baseline component of the study. Upon consideration of attrition rates commonly seen in

survey/questionnaire-based research, a response rate of 50% is often considered adequate, whilst 70% is deemed very good.[303] Other authors have suggested that researchers strive to limit maximum loss to follow-up to less than 20%.[304] Although the follow-up aspect of the present study follows a cohort study design, loss to follow-up rates of more than 20% in randomized controlled trials (RCT's) have also been shown to affect the validity of trial results.[305] Data published in 2013 shows that almost one-quarter of patients presenting with smear-positive sputum results are lost to follow-up before starting with their TB treatment.[306] Factors identified as having an impact on patients once treatment has commenced include socio-economic issues, patient specific factors, treatment-related issues and factors relating to the specific function of the health system in the country.[5]

With regard to demographic data, the majority of participants in the present study were males, which is in agreement with global data and data from the Western Cape Province.[18,23,24,36] This phenomenon has been widely documented in literature, and proposed theories include a mixture of socio-economic and cultural factors, including inequality on various platforms, thus affecting access to health care services and adherence to pharmacological treatment.[24,307,308] Other hypotheses for the disproportional gender findings include greater exposure of men to *M tuberculosis* as a result of increased opportunity for social interaction[309,310] and inter-gender variations in immunological processes and sexual hormones.[311,312] The impact of gender on treatment outcomes is also of interest but is unfortunately beyond the scope of this study.

The mean age of the study participants agreed with global data indicating that the majority of TB cases occur in the 15 to 59-year-old age group.[18] A further sub-analysis also showed that the majority of participants fell into the 18 to 30-year-old age group (young adults),[23] a vital segment of the national work force, which could have crippling effects on the functioning of the nation's economy.

The majority of participants were black African, followed by the coloured group and then the white participants. This is largely in agreement with the national demographic profile of South Africa, with black African's being in the majority (80.2%) and coloured and white participants being similarly distributed as 8.8% and 8.4% respectively.[313] However, given the strong coloured representation in the Western Cape Province, this group is very often in the majority, followed by black African's and then whites.[313] Possible reasons for the disparity in racial distribution could be clinic-specific because the area of data collection is known to be home to citizens of neighbouring African countries such as Zimbabwe, and these persons would be classified as black African.

4.2 ANTHROPOMETRY RESULTS

Group 1 and 2 were selected on the basis of consecutive sampling, and no significant differences were expected between the groups. If any differences did occur, it could probably be explained by chance.

Upon consideration of the significant mean anthropometrical measurements at baseline according to gender, males had both a higher body weight and height (stature). This occurrence is generally accepted since males have a higher body weight than females due to a larger stature and increased muscle (or fat-free) mass.[2] Conversely, a national survey in 2013, (SANHANES-1), conducted among the general population showed that whilst males tended to be significantly taller than females, the latter had a far higher body weight than the males.[301] Although this is not true for the present TB-specific study population, it is, however, evidence of the growing obesity epidemic in the country, especially among females because a larger body weight is often culturally linked to greater wealth and prosperity. Although the mean BMI of the female participants in this study was slightly higher than their male counterparts, this difference was not significant. Although TB-infected individuals do not typically present with an increased BMI, current evidence in South Africa shows that far more women fall into either the 'overweight' or 'obese' BMI category than do males.[301] It is, however, important to realise that the SANHANES-1 data was obtained from the general South African population, which differs vastly from the specific TB population under investigation.

Although the mean BMI at baseline was classified in the normal range for both males and females, there was an overall prevalence of 33.9% of undernutrition (classified as a BMI <18.5 kg/m^2) among the study population. These rates of undernutrition are in agreement with previously described rates of between 20% and 71.6%, albeit on the lower end of the spectrum.[59] A low BMI at diagnosis has been linked to an increased risk of relapse[61] and demonstrates an indirectly proportional relationship existing between BMI and mortality risk.[68] These at-risk patients should, therefore, be noted at diagnosis and followed-up throughout the treatment process.

Although the basic anthropometrical measurements are performed upon diagnosis (weight, height and BMI) in accordance with the Department of Health (DoH) guidelines, and the patient is placed on the Nutritional Therapeutic Programme (NTP) if need be, in South Africa, there are often many problems relating to the successful implementation thereof. In the personal experience of the researcher, these problems often equate to issues such as high patient default rates, unavailability of supplementation stock at facilities and general abuse of the programme such as sharing and substitution of products

between family members. Ultimately, these have a detrimental impact on the nutritional status of the individual. Of interest to this particular study is the fact that a small number of participants were entered onto the NTP by the clinic staff because they met the qualifying criteria mentioned previously. The provision of supplementation could, therefore, be seen as a contributing factor to the weight gain seen over time, although it is uncertain if these products were issued on a regular basis or used correctly by the patient.

Previous studies have also indicated that the occurrence of undernutrition among TB patients cannot be solely attributed to the disease itself but rather to a multitude of contributing factors, such as extreme poverty, food insecurity and reduced health-seeking behaviours.[67,69] Although South Africa is still viewed as a developing country, levels of socio-economic depletion may not be as crippling as in other African countries. The high rates of overweight and obesity found among the general population in South Africa could also have a spin-off effect of increasing the mean BMI of the average TB patient from undernourished to normal (as seen in the current study) when compared with other African countries where overnutrition is not as prevalent.

It was unfortunately beyond the scope of this study to assess TB-related weight loss before diagnosis and treatment commencement, and results would very likely be inaccurate if reliant on patient reporting alone. It would, however, be of interest to determine which patients were underweight for a time before their diagnosis, as well as track their progress whilst on treatment, since some studies have shown underweight to be an independent risk factor for TB disease.[61,62] The weight loss experienced in patients with TB is postulated to be part of the APR, resulting in the release of cytokines, leading to an overall anorexic effect.[114]

There were no significant differences in the measures of abdominal obesity between genders. According to the recent SANHANES-1 findings,[301] one might have expected to see the females with larger measurements. Since all participants were excluded on the basis of having an increased waist circumference measurement (>88 cm in females and >102 cm in males), it is, therefore, logical that all of the patients would fall into the ‘not at risk’ group. This is in contrast to the increased prevalence of augmented measurements in the general South African population, with approximately half of the female population having an enlarged waist circumference.[301] Given the fact that this TB study population was somewhat more prone to undernutrition than the general population and differed from the normal population profile, the results were, therefore, as expected.

More females than males were classified as having an increased waist:hip ratio (although the waist:hip ratio measurements showed no significant inter-gender differences), which was congruent with findings in the SANHANES-1 study. Waist circumference seems to be the more reliable indicator of visceral fat distribution.[2,161] Both the IDF and ATP III definitions are relying more on this measurement than the previous waist:hip ratio, which is postulated not to be as absolute a measurement.[180] Current thinking also regards fat distribution (i.e. android vs. gynoid) to be a more valuable risk factor for mortality and morbidity than obesity (i.e. measured by BMI).[2]

The female participants also presented with significantly higher measurements of skinfolds, fat mass and percentage body fat. These findings are in agreement with the literature in which the ranges of cut-off for body fat for women are significantly higher than those for males.[2] The higher fat mass in females also indicates why conversely, males have a higher fat-free mass.

Upon consideration of the percentage body fat of the participants, the majority fell into the groups of ‘underweight’ (33.9%) and ‘slim’ (50.8%). This links to the concept of TB wasting in which a loss of both fat mass and lean body mass (LBM) has been described in similar patients.[55] Although the current study excluded HIV-infected persons, previous studies investigating wasting in HIV-infected and uninfected TB patients have postulated that TB, not HIV, seems to be the main driving force behind the wasting occurrence.[55]

Previous studies have documented the seemingly greater loss of LBM in the limbs compared with the FM loss in the trunk area due to the TB wasting syndrome.[55] Regarding this study, if one considered the triceps skinfold (peripheral fat) as well as the subscapular skinfold (truncal fat), it was noted that whilst the triceps values for both genders were interpreted as being below average, the subscapular skinfold in the female group was defined as average, thereby not reflecting a decreased FM in this area. This occurrence should ideally be repeated among a larger study population to assess the accuracy of findings in the current study. This also highlights the possibility of gender-specific differences in body composition in TB patients, as previously reported by Mupere et al.[81]

Because the participants were not stratified according to demographics (gender, race or age), possible conclusions were interpreted with caution.

Markers of abdominal obesity performed in accordance with the literature since both waist circumference and waist:hip ratio increased with age, as was documented in the SANHANES-1

study.[301] Although the oldest participant in the current study was only 62 years old, it was noted that body weight,[314] total body fat and intra-abdominal fat generally increase with age, very often at the expense of subcutaneous peripheral fat.[2]

The mean weight of the participants in the follow-up group increased by 7.4% overall between baseline and five months. The greatest weight gain occurred during the intensive phase of treatment, which was synonymous with the majority of the findings in this study showing significance during that specific time period. The intensive phase is described as the initial two months after treatment commencement, when a more aggressive regimen of TB drugs was prescribed, namely isoniazid, rifampicin, ethambutol and pyrazinamide.[5] This regimen was largely followed in order to reduce the possibility of developing drug resistance and to target the various strains of bacteria.[21] Once this period was completed, a less antagonistic protocol was followed in which fewer drugs were administered.

This finding regarding the period of weight gain is interesting since one could expect a catabolic effect accompanying infectious conditions, which may have an inhibitory effect on weight gain. Evidence of the catabolic effect was seen in a study conducted in Cape Town, South Africa that documented an initial weight loss amongst hospitalised (severely ill) HIV-negative TB patients shortly after treatment commencement.[315]

It could also be postulated that this catabolic stage could occur pre-diagnosis when the patient is still largely symptomatic. Therefore, after TB treatment commencement and symptom resolution, patients could undergo an improvement in clinical symptoms, such as an increase in appetite, which could partially be responsible for the weight gain seen during this time. Of the ten patients that experienced a weight loss during the current study, the minority (30%) underwent the loss during the first two months. This could perhaps be equated to the patients in this study being ambulatory who were well enough to attend the health facility, versus those who were more severely ill.

Overall weight gain of TB patients whilst on treatment is also a common phenomenon [54-56,71,73-76] although some researchers are hesitant to use weight gain as a marker of successful response to treatment.[66,316] Although the majority of patients gained weight over the duration of treatment, there were some that lost weight at two or five months. All participants, bar one, had surpassed their baseline weights by the end of the follow-up period.

It has been reported that patients with a low BMI at diagnosis and those failing to gain more than 5% weight between diagnosis and completion of treatment were at greater risk of relapse.[61] In the current study population, 31.0% of participants did not achieve more than 5% overall weight gain. The majority of these patients all had normal BMI's at the end of treatment, which may cause them to be overlooked if BMI is used as the sole anthropometric predictor of treatment outcome. These patients should, therefore, be observed closely and routinely followed-up at the health facility, even after successful anti-tuberculosis treatment.

Of the patients who were classified as underweight according to BMI at baseline, there were several that remained in this category at the five-month follow-up despite having gained weight. This is unfortunately also a common occurrence in this particular patient group[66,71] and once again highlights the need for follow-up and support post-treatment. There were three patients who fell into the upper range of normal BMI at baseline but were subsequently classified as overweight at two and five months due to weight gain. Therefore, attention should also be given to these patients because they are considered malnourished and could be overlooked in light of their leaner counterparts.

There was a significant increase in measures of abdominal adiposity over the five-month period. There was only one participant who exceeded the previously mentioned cut-off points for waist circumference at two and five months, thus placing him at a higher risk of developing chronic diseases. However, the overall increase seen in the study population should still be monitored given the excessive visceral obesity rates seen in South Africa and the propensity of gains in fat mass whilst on TB treatment.[55]

Because the BMI and body weight alone are often insufficient means for documenting changes in body composition,[81] skinfolds were also performed in the current study. Previous research conducted by Paton et al. in 2004 reported that the majority of later weight gain in TB patients on treatment was due to an increase in fat mass (FM).[57] Results of the current study, however, show a significant increase in fat free mass (FFM) in all follow-up periods, which is in contrast with the increase in fat mass seen only during the intensive phase of treatment and between baseline and five months. This might indicate that patients in this study gained FFM beyond the intensive two-month period, which is promising because LBM or protein mass is associated with improved functionality, quality of life and longevity.[57,66,84]

With regard to the sum of skinfolds measurement, findings largely echoed that of the fat mass and percentage body fat measurement, with the noticeable differences occurring during the intensive phase of treatment. The peripheral skinfold measurements (biceps and triceps) either showed no differences

over time or the increases occurred after the two-month period. The trunk/central measurements, namely the suprailiac skinfold, mimicked the sum of skinfolds outcome, whilst the subscapular values showed an increase only at the five-month mark. Gains in FM, therefore, seemed to occur in both the peripheral and trunk area of these patients (albeit slightly more in the trunk area), resulting in a more proportional fat distribution. Although there are individual variations in each of the four skinfolds measured, participants in this study experienced an overall significant increase in fat mass whilst on TB treatment, which is in line with the literature.

The secondary objective of assessing any changes in anthropometrics and body composition between baseline and the follow-up periods was thus achieved since significant differences were noted.

4.3 BIOCHEMISTRY RESULTS

The results obtained from study participants were all compared with standardised values used by the laboratory tasked in the analysis of the specimens (NHLS).[289]

Albumin and CRP are both markers of the inflammatory response, the former a negative acute phase protein, whilst the latter is a positive acute phase protein. If considering the baseline albumin levels of the study population, the mean albumin level was within normal reference ranges. Contrary to this finding, the mean CRP level at baseline was noticeably raised. Findings verifying low levels of albumin and high CRP levels in TB patients have been well documented in literature. [52,114,120,122-124] However, it is perhaps the raised CRP level that is more indicative of active TB disease because it has been described as a non-specific measure of systemic inflammation in the body.[114] The degree of CRP escalation has also been linked to the presence of weight loss and to disease severity, and this may increase levels even further.[114]

No hospitalised, drug-resistant or HIV-infected patients were included in this study. Therefore, the albumin levels were possibly not as severely affected due to the patients being relatively ‘well’ TB patients (i.e. with predominantly normal BMI’s). This could perhaps also be equated to a form of ‘adult marasmus’, a form of PEM, whereby the albumin level remains normal.[317] One could postulate that in more stressed, hospitalised patients, the albumin level may be significantly lower; a study in South America associated on-admission hypoalbuminaemic levels in TB patients with an increased risk of in-hospital mortality.[120] Hypoalbuminaemia is also linked to the inhibitory action of cytokines on albumin

production (due to albumin being a negative acute phase protein), ultimately lowering levels of the protein and resulting in alterations in plasma concentrations of other essential micronutrients.[111]

Despite the mean white cell count (WCC), also referred to as the leukocyte count, falling into the normal reference range, about one-third of the study participants presented with increased values. This occurrence is relatively common because increased white cells (leukocytosis) often indicate the presence of an infection in the body, which is the case in TB. Incidences of leukocytosis in the setting of TB compared with healthy controls have also been described in the literature.[72,114]

According to the SEMSDA 2012 guidelines for the management of type 2 diabetes, a fasting plasma glucose level of between 6.1 mmol/L and 6.9 mmol/L classifies an individual with impaired fasting glucose (IFG) and any value ≥7.0 mmol/L to be indicative of a diabetes diagnosis.[3] Taking this into account, only one individual in the current study was classified with IFG (1.7%) and two with diabetes (3.4%). The two patients who were classified as having diabetes on the basis of their baseline fasting glucose result were, however, not excluded from participating in the study because they were not diagnosed diabetics at the time of recruitment (See Chapter 2.5). A systematic review published by Jeon et al. in 2010 showed total DM prevalence in TB patients to fall between the range of 1.9% and 35%,[251] but some of these were diagnosed only after development of TB. Given South Africa's high prevalence rates of TB, its status as one of 22 HBCs and its high rate of non-communicable diseases, this study may have been expected to exhibit a far higher number of DM patients.[243] However, it is important to remember that participants recruited for this study excluded any persons already diagnosed with diabetes, which resulted in a lower prevalence rate.

Upon consideration of the minority of patients that were classified as having either IFG or diabetes on the basis of their fasting glucose levels at baseline, it is prudent to make comparisons with similar studies. In a cross-sectional study conducted in West Africa, participants displayed a 5% IFG and a 1.9% DM rate (in accordance with SEMSDA cut-off points above), which is comparable with the current study despite differing inclusion criteria (inclusion of pre-existing diabetics, HIV-positive and/or obese patients in the West-African study sample).[318] A retrospective study performed in Sri Lanka found 7.1% pre-existing DM among their study sample, as well as 20% IFG and 2% DM rates. The high rate of IFG found in this study could perhaps be attributed to the lower fasting glucose cut-off points used in the diagnosis of this condition (5.6 – 6.9 mmol/L).[319]

Therefore, it would be advised to repeat the fasting plasma glucose in those patients with either IFG or suspected DM and to perform another corroborative test, such as an OGTT or HbA1C, before diagnosing diabetes. This stress/transient hyperglycaemia experienced in TB patients is a well-described phenomenon in the literature since the raised glucose levels at diagnosis[246,251,254] often subside with the progression of treatment.[248,249] Certain anti-TB medications (such as Rifampicin) have also been documented to result in higher than normal levels of glucose in the beginning stages of treatment.[260] In addition, the effect of pro-inflammatory cytokines is possibly related to IR increase and concomitant reduced insulin production.[244] Counter-regulatory stress hormones (e.g. cortisol, glucagon and epinephrine) are often released during periods of inflammation in the body, and these have been shown to inhibit insulin action, which could also result in the hyperglycaemic response.[245] These phenomena highlight the importance of confirmatory DM tests at diagnosis, during treatment and post-treatment.[243]

The preliminary stages of IR often present with normal fasting glucose levels (contrary to the TB medication-induced hyperglycaemia), which will progress to impaired fasting glucose and ultimately DM as the condition develops. This could indicate that patients with a normal glucose at baseline have already suffered β-cell damage and could have IR.[320]

Although an isolated fasting insulin measurement was previously credited with being a relatively good indicator of IR,[214] given the pathophysiology of IR and hyperinsulinaemia, current opinion suggests that due to the variability of the marker, formulae encompassing more than one fasting value are preferred, such as the HOMA-IR or QUICKI.[194] Although normal reference values for fasting insulin do exist, there are no standardised cut-off values specifically identifying IR. The current study showed a very small proportion of patients having raised insulin levels at baseline, thereby confirming the suggestion that fasting insulin alone is insufficient to classify IR.

A study conducted in the Eastern Cape Province of South Africa found higher levels of both glucose and insulin in TB patients compared with controls.[257] Once again, due to the unavailability of a control group in this particular study, it is difficult to make comparisons. It could be postulated that although the TB patients in this study did not show abnormally high levels of glucose or insulin (according to the cut-off values used), the levels could perhaps be higher than in those who did not have active TB disease. The findings from the Eastern Cape study also suggested that there was an alteration in insulin/glucose metabolism in the patients with TB, which is in agreement with the suggestion of Karachunskii et al. that carbohydrate metabolism was altered.[258]

With regard to the lipogram performed at baseline, the most noticeable abnormality was the reduced HDL-cholesterol values, which occurred in the majority of patients. A lowered total cholesterol level has previously been described in the literature,[45,113,114] in which it was hypothesised that prolonged persistence of the TB bacterium may result in cholesterol breakdown, especially in persons who had been infected latently for a period of time. Interestingly, many of the conditions that are believed to result in higher TB risk, often present with concomitant hypercholesterolaemia.[117]

Reduced HDL levels have been reported in numerous studies with various infectious or inflammatory conditions.[321-323] A study by Deniz et al. in 2007 showed lowered levels of HDL-cholesterol in their PTB-specific study population.[113] The authors hypothesised that this finding could be linked to the activated APR often seen in TB disease and, more specifically, to acute phase proteins, secretory phospholipase A2 (sPLA2) and serum amyloid A (SAA).[324-327] Other compounds such as ATP-binding cassette transporter (ABC)-1 and decreased lecithin cholesterol acyltransferase have also been implicated in reduced HDL-cholesterol during periods of infection or inflammation, albeit some in animal studies. [328,329] The Deniz et al. study also showed that the HDL-cholesterol concentrations were negatively correlated with the degree of smear positivity as well as the radiological extent of the disease, therefore, identifying the 'sicker' patients with lowered levels of HDL-cholesterol.[113] In addition, low HDL levels have also been linked to an increased cardiovascular disease risk.[301]

Previously, LDL-cholesterol levels have also shown to be diminished in times of infection or inflammation,[113] largely as a result of activated host response and LDL oxidation.[322] If one had to speculate and compare findings from this study to the general population, as found in the SANHANES-1 study in 2013, levels of total cholesterol, LDL-and HDL-cholesterol as well as triglycerides for both genders were all lower than the mean values in the larger national cohort.[301] Once more, this finding could be expected because this was a very specific study population (obese and metabolic syndrome patients excluded). In a population consisting of chronic diseases of lifestyle participants (obesity and metabolic syndrome included), one might expect to see higher levels of blood lipids.

The current study found a significant difference between inter-gender total cholesterol levels, with females showing a higher value. This is in line with findings from the SANHANES-1 study that found higher levels of total and LDL-cholesterol when compared with males.[301] Literature has also documented findings of lower triglycerides and higher HDL-cholesterol in women[330-334] although no significance was found in this study. On the contrary, other studies have found males to have a more atherogenic profile

than their female counterparts.[335] It should be noted, however, that since the study was not stratified according to demographics, this data should be interpreted with caution.

With regard to racial distribution, the current study found that the white participants had the highest levels of total and LDL-cholesterol, whereas the black African group displayed the lowest levels. This finding is in line with SANHANES-1 data, which also placed the black African race in the more 'beneficial' group.[301] This can be further extrapolated because epidemiologically, black African males tend to have a greater prevalence of cardiovascular risk factors, yet their lipograms reflect a more positive HDL,[331,332,336-339] LDL[336,337]and triglyceride[331-333,336-339] picture when compared with white males.[340] Whilst literature has reported black or African American males to have higher HDL-levels due to possible reduced activity of hepatic lipase,[341] the current study shows the HDL levels to be lower among the black African population.

The baseline fasting insulin level was shown to be significantly higher among younger participants. See Chapter 4.5 for further discussion on this phenomenon.

Upon consideration of follow-up biochemisry results, the majority of changes occurred during the intensive phase of treatment (i.e. from baseline to two months after commencement of treatment). During this time, a heightened response to the treatment is often noticed, depending on the patient's adherence to the medication. This reduction in mycobacterial load has also been documented in previous years by the WHO, which noticed an improvement over the first three months of treatment.[342] Although the majority of significant results are seen in this time period, it remains vital to complete the full course of treatment because the intensive phase cannot singularly resolve TB.[107] In the current study, albumin, CRP, white cell count, total, LDL-and HDL-cholesterol all experienced significant changes during this specific time period.

Although there were two participants who could be classified as having 'diabetes' according to their fasting glucose levels at baseline, there were no patients with significantly increased levels at either two or five months. Mean glucose levels were reduced (although not significantly) between baseline and five months, and this could perhaps be seen as a resolution of the hyperglycaemic response seen in the beginning stages of treatment. This has been previously described in a few studies.[246,248] The reduced blood glucose levels could also be attributed to the diminishing infection over time.[254] This phenomenon could possibly be explained by the diminished effect of the anti-TB treatment, notably rifampicin, because this pharmaceutical aid has been shown to induce a maximum hyperglycaemic

effect approximately seven days after commencement of treatment, which resolves two weeks after ceasing treatment.[343]

As previously mentioned, albumin and CRP are both synonymous with the APR, and TB can be seen to elicit a similar response in the body. The current study saw an increase in albumin levels, as well as a decrease in CRP. This would suggest a 'resolution' of the APR or a suppression of the inflammatory response,[122] which is a phenomenon well reported in literature, both for albumin[107] and CRP.[46,107,122-126] The white cell count performed similarly to the CRP, with a significant decrease between baseline and two months. This could also be indicative of an improved response to infection or inflammation although mean values remained normal throughout the five month period, which could indicate that CRP is the more sensitive marker.

In the follow-up group, the majority of patients had a raised CRP level at baseline although this decreased over two and five months. There were, however, some patients who still presented with a raised CRP at the five-month mark, which could perhaps indicate a sub-optimal treatment response[123] or the presence of other infections, especially if the CRP did not show an overall downward trend. The elevated CRP together with an overall clinical picture of the patient should, therefore, be considered, and these patients should be monitored closely for treatment failure or possible relapse risk.

The mean albumin level of the follow-up group remained within the normal range throughout, and there was only one patient who had a reduced albumin level at the five-month mark. This is in contrast with the raised CRP levels, which remained high even at five months, as well as there being eleven participants with raised levels at this time period. This could perhaps suggest that CRP could be a better marker of treatment response when compared with albumin.

The phenomenon of reduced total and HDL-cholesterol levels at baseline has already been discussed in Chapter 4.3. Total cholesterol, as well as LDL-and HDL-cholesterol all experienced a significant increase in the intensive treatment phase, with no significant differences occurring between the two-and five-month periods. This is an unusual finding because one might expect the values to increase even more at the end of the follow-up period. This finding could be the result of a small sample size and definitely warrants more investigation given the important role of adequate cholesterol levels in protection against the mycobacteria.

A very controversial recommendation based on the hypothesis that an increased dietary intake of cholesterol or cholesterol supplementation, could perhaps fast-track the smear-conversion rate in PTB patients, has been made.[344,345] If future findings show that cholesterol levels experience a tapering off after the intensive phase, these researchers suggest the possibility of exploring the option of exogenous cholesterol provision as a prophylactic measure to improve treatment response and relapse risk.[344,345] This is still, however, in a very exploratory stage and should not be recommended as standard practice since more evidence is needed.

The secondary objective of assessing changes in the biochemistry values between baseline and the follow-up periods was also achieved, and significant differences were noted.

4.4 BLOOD PRESSURE RESULTS

Given the importance of hypertension as a risk factor for the development of cardiovascular disease (including stroke and ischemic heart disease), it was of interest to consider the prevalence in this particular population because South Africa suffers from a high burden of non-communicable diseases. The presence of hypertension (raised levels and/or on medication) is a category in assessing the metabolic syndrome, in which the presence of IR is strongly associated.

The minority of participants had a blood pressure value that classified them as hypertensive at baseline (13.6%), and 18.7% were defined as pre-hypertensive. The majority of participants fell into the normal blood pressure category. These results should, however, be interpreted with caution because hypertension is not traditionally diagnosed during the first visit (as was done in the current study) but rather over a period of time.[346]

The prevalence rate found in this study is noticeably lower than global data published in 2001, which showed a worldwide hypertension prevalence of 26.4%,[347] and specific South African data generated from a survey performed in 1998 by Steyn et al. indicated South Africa had a minimally lower prevalence rate of 23.9%.[348] Results from the Lancet study also make mention of the increased risk of hypertension in developing countries, of which South Africa is one.[347] More recently, blood pressure values in South Africa were clinically assessed by the SANHANES-1 group on a small sub-sample of participants and showed pre-hypertensive rates of 10.4% in persons aged 15 and older, whilst hypertensive rates depicted a 10.2% nationwide prevalence.[301] These results should, however, be interpreted with caution

because a large proportion of the blood pressure data in the SANHANES-1 study was self-reported. Once more, it is challenging to apply the available hypertension prevalence rates to the study population because it differs considerably from the general population from which the majority of data was gathered.

There were no significant differences noted between baseline and the follow-up periods for blood pressure specifically, which addresses another of the secondary objectives (See Chapter 2.1).

4.5 DIAGNOSTIC INSULIN RESISTANCE TEST RESULTS

As was mentioned in Chapter 1.5, a multitude of different methods exist for the measurement and diagnosis of an IR state. Although the hyperinsulinaemic-euglycaemic clamp (HEC) has largely been recognised as the 'gold standard',[6,13,187,188] it is not without its disadvantages (often pertaining to excessive cost and labour constraints), and less invasive indices are, therefore, often utilised.

The HOMA-IR is one of the so-called 'fasting indices', which utilises fasting measurements of both glucose and insulin in its formula calculation. The HOMA-IR has been used extensively in epidemiological research and as a routine measurement in clinical practice[187] and together with the QUICKI[20] has proved to be a very popular fasting index.[7] It has shown positive correlations with the gold standard measurement (HEC),[13,187,196,216] been validated by the Insulin Resistance and Atherosclerosis study (IRAS)[221] and has also been recommended as one of the methods for IR testing by the IDF.[180] The HOMA-IR is simple to perform, not labour intensive for staff, minimally invasive for the patient, cost-effective and useful in follow-up studies.[6,10,13,142,189] This made it an appropriate choice for the current study although it is definitely not without criticism due to several limitations of fasting indices in general, such as measuring hepatic and not peripheral IR.

Newer versions of the HOMA-IR are mentioned in literature such as the HOMA2, which was released in 2004 and details the β-cell function and insulin sensitivity of an individual.[13] This version has, however, not been validated[196] and according to the literature is not used widely for the classification of IR. It was thus not used in the current study. The majority of other studies using the HOMA as a reference value have used the HOMA-IR, which makes inter-study comparison more feasible.

Although no significant results emerged from this sub-section (apart from IR tests and age), it was noted that the mean baseline value for HOMA-IR was above the cut-off point used to classify IR in this study. Similarly, the calculated QUICKI cut-off was below the mean value, thus also classifying IR. There were no significant differences for either gender or race but again, caution should be exercised given the lack of stratification, which fell outside the scope of this study. Various sources have identified age, gender and race or ethnicity-specific differences in HOMA-IR and QUICKI levels, and specifically in South Africa, greater levels of IR have been found among obese black Africans and Asian Indians when compared with white persons.[349-350] This would be useful to pursue in a follow-up study seeking to document these specific occurrences.

The incidence of IR decreased (lower HOMA-IR and higher QUICKI) with age, as was seen previously with a significantly higher baseline fasting insulin value among the younger participants. This phenomenon is discussed in more detail in Chapter 4.8.

The EPIRCE study conducted in Spain also showed HOMA-IR levels to be influenced by physical activity, alcohol intake and smoking.[351] Whilst physical activity had a protective action by reducing HOMA-IR levels, abstaining from substances such as alcohol and nicotine did not since abstainers were shown to have higher HOMA-IR levels for both substances.[351] Substance abuse, namely alcohol intake of >40 g per day and smoking, are responsible for increasing TB risk by a relative risk of 2.9 and 2.0 respectively.[352]

Recent research has also indicated the possibility of genetic influences on the development of IR, although at the present moment, minimal variants have been associated with IR development. An example of this has been identified as a variant of the N-acetyltransferase 2 (NAT2) gene which has been linked to decreased insulin sensitivity irrespective of BMI.[353] These genetic studies are, however, still in the preliminary stages of investigation but could potentially explain some of the current study participant's having IR despite the absence of the typical chronic disease phenotype.

The follow-up profiles of both HOMA-IR and QUICKI seemed to indicate an improvement in IR over time. Although there were no significant differences noted between any of the time frames, there was an overall downward pattern with the HOMA-IR and an upward curve for the QUICKI, both signifying a 'lessening' of the IR state. The lack of significance could perhaps be due to the relatively small sample size. However, this ties in closely with an improved and diminishing inflammatory response, as demonstrated with the improved CRP, albumin and white cell count discussed previously. This could

also be due to the well-described 'stress or transient' hyperglycaemia seen to occur in the early stages after TB diagnosis and which often resolves with progression of treatment.[242,248,249] A state of hyperglycaemia would either increase the HOMA-IR value, or decrease the QUICKI reading, thus indicating an increased risk for IR.

Despite the largely improved IR status over the five-month follow-up period and the majority of patients at the beginning stages of the study having resolved their IR status (albeit two participants), the following was noted. Two participants who had normal HOMA-IR and QUICKI values at baseline subsequently developed IR at two and five months, and two patients redeveloped IR at five months after originally presenting with it at baseline. This contests the stress hyperglyacemia hypothesis, as well as the improvement of an inflammatory state, and is an interesting phenomenon to be pursued. It would perhaps be prudent to follow-up such participants at a later stage to re-determine the IR status once the TB has resolved.

An assessment of changes seen in IR status (using the HOMA-IR and QUICKI) was, therefore, documented and although no significant results were recorded, this secondary objective was adequately addressed.

4.6 METABOLIC SYNDROME INVESTIGATION

Due to the lack of consensus regarding current guidelines for the diagnosis of metabolic syndrome, several studies have made use of multiple criteria to classify their particular study populations.[354-356] Some of the most popular include the IDF and ATP III guidelines, which were the two tools used in the current study.

Considering the prevalence of individual components of the syndrome among the total study population at baseline, the following was shown: increased waist circumference (5.1% for IDF and 0.0% for ATP III); raised triglycerides (1.7% for both IDF and ATP III); reduced HDL-cholesterol (69.5% for both IDF and ATP III); increased blood pressure (13.6% for both IDF and ATP III); and raised fasting plasma glucose (6.8% for both IDF and ATP III). Apart from the reduced HDL-cholesterol, which has previously been discussed within the context of TB (Chapter 4.3), this corroborates the relatively low prevalence of individual metabolic syndrome findings, which is to be expected among this study population.

Two participants were classified by the IDF criteria at baseline (3.4%), whereas only one was classified by the ATP III criteria (1.7%). This seems to indicate that the IDF is a more specific indicator for IR. However, in the group of patients that were followed up, the ATP III criteria identified one person with the syndrome at two months, which the IDF did not. This is contrary to the baseline findings and highlights the disparity between the two methods. This disparity has also been shown in large studies such as the AusDiab and DECODE studies, in which the former found that less than 10% of patients met the criteria for each of the IDF, ATP III and WHO guidelines. [357,358] This also makes inter-study comparison challenging because data is not standardised according to a single definition.

The major difference between the IDF and ATP III guidelines is the classification of the waist circumference because different cut-offs are employed. In addition, an increased measurement is a prerequisite for the IDF criteria. The ATP III criteria do not require pre-requisite factors in the classification of metabolic syndrome and have been reported to have a higher sensitivity than the IDF classification.[359] Although IR is recognised as an important facet of the metabolic syndrome, both the IDF and ATP III guidelines do not take IR into account when classifying an individual with the syndrome, which is in contrast to previous definitions (e.g. WHO). This is mainly due to the lack of standardised reference values for IR and problems with inter-study measurements of insulin.[360] The above-mentioned disparity between the different criteria highlights the need to develop a standardised set of guidelines that are country and ethnicity specific and are able to identify all individuals correctly. Until this time, the most suitable tool (or combination of tools) and cut-off values should be utilised.

When compared with the suggested global prevalence of metabolic syndrome that ranges between 15% and 40%,[9,147,148] the prevalence rates found at baseline in this study (3.4%) are much lower, which could be viewed positively. This could be expected, given the exclusion of patients presenting with typical ‘metabolic syndrome’ during the recruitment process. The low prevalence rates in this population can also be viewed in the light of findings from other studies that indicate a much lower prevalence of the syndrome among normal weight or overweight subjects when compared with obese individuals,[356,361,362] of which the current study had none. One could also expect a lower prevalence of the syndrome among underweight individuals, of which there were several in this study.

Of the patients classified as having IR according to an increased HOMA-IR level (>2.477), only one was identified as having the metabolic syndrome according to both the IDF and ATP III criteria at baseline. One IR participant identified by the ATP III criteria presented with the syndrome at two months, whereas no IR patients met either the IDF or ATP III criteria at the five-month follow-up. Given the increased

prevalence of IR at baseline, according to the specific HOMA-IR cut-off used (25.4%), it appears that IR is present before the development of the other components of the metabolic syndrome, and is a result of a longstanding IR state in the body.[172,363] The HOMA-IR may possibly have preventatively identified at-risk persons who may have been overlooked by the IDF and ATP III criteria.[354] In a study assessing the relationship between IR diagnosis and the ATP III criteria, it was found that the ATP III guidelines do not show good sensitivity in identifying individuals with IR.[219] The SuRFNCD-2007 study also found the presence of IR in approximately one-third of participants who were not identified by either the ATP III or IDF criteria.[364] It would be ideal if one could target patients with suspected IR before the onset of the metabolic syndrome via lifestyle changes (diet and exercise) and thus prevent the cascade of complications from being triggered, although some researchers argue to the contrary.[219]

It has previously been mentioned that due to the limitations of the fasting indices (such as HOMA-IR or QUICKI), it is often preferable to combine additional parameters when assessing IR status. These parameters include classic risk factors such as waist circumference, blood pressure and lipid profile and should be viewed in light of an abnormal fasting index result.[13,187] Although this is the approach often taken by many researchers and certain laboratories in South Africa, it may be prudent to consider the fasting index alone in a non-typical metabolic syndrome population (i.e. TB), given the disparity in identification of at-risk persons.

When comparing the HOMA-IR with all of the individual criteria for metabolic syndrome, only the fasting glucose was found to show a significant positive correlation. This is logical because the fasting glucose is a factor used in the calculation formula of the HOMA-IR (see Chapter 2.6.3.4 for the specific formula). Although there were no other significant results found, an increasing HOMA-IR was found with increased triglycerides and blood pressure, whilst HDL was lower with increasing HOMA-IR. This would seem to be in line with the metabolic syndrome criteria discussed above and is definitely an avenue worth investigating with repeated research and a larger sample size.

The primary objective of the study was to determine whether IR was present in PTB patients, through the use of the HOMA-IR and QUICKI, as well as through anthropometrical and biochemical markers. Since anthropometry (waist circumference), biochemistry (HDL-cholesterol, triglycerides and fasting glucose) and blood pressure measurements formed part of the assessment for metabolic syndrome, this objective (together with diagnostic IR tools) has been achieved.

4.7 DETERMINATION OF HOMA-IR CUT-OFF POINT

As mentioned in Chapter 1, there is currently no consensus as to the HOMA-IR cut-off points used to classify IR, which makes comparison between studies challenging.[364] Previous researchers have made use of ROC curves to estimate HOMA-IR cut-offs,[296,365] whilst others have used the Youden index and distance from the top left-hand corner of the ROC curve to quantify cut-offs.[364] Another method employed is to calculate the value of either the median,[366-370] 75th percentile,[293-296] 90th percentile,[371-374] lower limit of the top quintile (80th percentile)[146,365] or tertile (66th percentile)[219,375,376] in non-obese subjects with no apparent metabolic disorders. Table 4.1 documents previous studies and their HOMA-IR cut-offs, as well as the comparison between those cut-offs and that of the current study.

Due to the disparity in defining a suitable HOMA-IR cut-off point, the current study employed the HOMA-IR cut-off value as the lower limit of the upper quartile (75th percentile) in this study population of TB patients (see Table 4.1). Previous studies have quantified IR to occur between the HOMA-IR levels of 2.1 and 3.8,[7,193] which is a vast range but in which the value of 2.477 calculated in this study fits comfortably. The median for this study was 1.394, which is noticeably lower than the proposed range of 2.1 – 3.8 mentioned above.

A South African laboratory practising outside of the Western Cape Province (Lancet Laboratories) performs the HOMA-IR index and states that a normal range for this variable is between 0.08 and 2.0.[377] They do, however, mention the uncertainty of the HOMA measurement in routine clinical practice and utilise the additional cut-off points provided by Stern et al. [171] (Table 4.1) as further diagnostic IR information. They also mention that the HOMA-IR and the QUICKI can be unreliable in cases of raised fasting glucose (>6.0 mmol/L).[171]

The variability seen in the HOMA-IR cut-off values seen in Table 4.1 could be due to the lack of standardised insulin assay and the variability in the types of population studied, thus making comparison between studies difficult. The current study is, to the knowledge of the author, the first of its kind to assess IR prevalence using a calculated HOMA-IR and QUICKI value in a population of TB patients.

The current study yielded an IR prevalence rate at baseline of 25.4% (Males=25.0%; females=27.3%), which is in line with findings in the general population of between 20% and 40%,[145,146] although some studies have placed the IR prevalence slightly lower (10 - 25%).[320] Although this finding may seem unexpectedly high, given the somewhat opposing clinical picture of TB and metabolic syndrome, recent

studies have reported high levels of hyperglycaemia, impaired glucose tolerance and DM in TB patients.[242,244,246-249,251,254,257-259,261-264] Glucose intolerance was also recently found to occur at rates of 34.2% and 18.3% for males and females respectively,[259] which indicates the increasing prevalence of this phenomenon and as such, formed the basis for this exploratory research study. The prevalence rate of IR according to the HOMA-IR levels in this study also showed a decreasing pattern (i.e. diminishing IR status with treatment progression), which was explained in Chapter 4.5.

The primary objective of the study was to assess if IR was present in patients with PTB by means of various tests, of which the main diagnostic tool was the HOMA-IR, with the QUICKI used as a confirmatory measurement. The current study has thus achieved this objective because a total prevalence of 25.4% according to both tools was found.

Table 4.1: Comparison of previous studies investigating HOMA-IR levels

Study	Population investigated	HOMA-IR cut-off value	Comparison with current study
Matthews et al. (1985)[7]	General population	Normal subjects = 1.21 – 1.45 IR diabetic subjects = 2.61 – 2.89	Lower than current study
Haffner et al. [San-Antonio study (1997)][378]	General population	9.5 in NIDDM 2.7 in normal glucose tolerance	Slightly higher than current study
Matsumoto et al. (1997)[374]	15 – 70 years (Japan) Non-diabetic and BMI <25 kg/m^2	90th percentile = 1.9	Lower than current study
Bonora et al. [Bruneck study (1998)][146]	40 - 79 years (Italy) No metabolic abnormalities (BMI <25 kg/m^2) Diabetic and non-diabetic	 80th percentile = 2.77 50th percentile = 2.5 and 75th percentile = 3.7	Slightly higher than current study
Yeni-Komshian et al. (2000)[367]	Healthy non-diabetic volunteers	2.7	Slightly higher than current study
Hedblad et al. (2000)[293]	45 – 64 years (Sweden) Non-diabetic individuals	75th percentile = 2.0	Lower than current study
Ascaso et al. (2001)[373]	20 – 65 years (Spain) No metabolic abnormalities	90th percentile = 3.8	Higher than current study
Marques-Vidal et al. (2002)[294]	35 – 64 years (France) Diabetic and non-diabetic	75th percentile = 3.8	Higher than current study
Nakai et al. (2002)[372]	Japan No metabolic abnormalities (BMI <25 kg/m^2)	90th percentile = 1.8	Lower than current study
Ascaso et al. (2003)[295]	30 – 60 years Hospital personnel (healthy subjects)	75th percentile = 2.6	Slightly higher than current study
Gokcel et al. (2003)[379]	15 – 87 years (Turkey) Healthy subjects	2.24 (SD 1.26)	Slightly lower than current study
Stern et al. (2005)[171]	General population	HOMA >4.65 or HOMA >3.60 and BMI >27.5 kg/m^2 or BMI >28.9 kg/m^2	Higher (but these cut-offs do not make allowance for non-overweight/obese subjects)
Geloneze et al. (2006)[371]	Brazil No metabolic abnormalities (BMI <2 5kg/m^2)	90th percentile = 2.7	Slightly higher than current study
Lee et al. (2006)[296]	30 – 79 years (Korea) Non-diabetic individuals	75th percentile = 3.04 Cut-off for increasing metabolic syndrome: HOMA-IR = 2.34 (ATP III criteria) HOMA-IR = 2.38 (IDF criteria)	Higher than current study (75th percentile)

Study	Population investigated	HOMA-IR cut-off value	Comparison with current study
Radikova et al. (2006)[380]	White rural population No previous history of diabetes or dysglycaemia	75th percentile = 2.29	Lower than current study
Bertoni et al. (2007)[368]	45 – 84 years (United States) Non-diabetic; multi-ethnicity	50th percentile = 1.2	Lower than current study
Sumner et al. NHANES (2008)[375]	≥20 years Non-diabetic adults in the United States Multi-ethnic study population	66th percentile = 2.73 75th percentile = 3.23	Slightly lower than 66th and lower than the 75th percentile
De Luis et al. (2009)[368]	Non-diabetic (Spain) Obese (BMI >30 kg/m^2)	50th percentile = 4.4 (SD 4.1)	Lower (population differed)
Esteghamati et al. (2009)[365]	20 – 77 years (Iran) No metabolic abnormalities (BMI <25 kg/m^2) Non-diabetic and non-hypertensive	50th percentile = 1.4 (SD 0.9) 75th percentile = 1.6 80th percentile = 1.8 90th percentile = 2.3 50th percentile = 1.8 (SD 1.2) 75th percentile = 2.3 80th percentile = 2.6 90th percentile = 3.3	Lower than current study (80th percentile) Similar to 75th and 80th percentiles
Esteghamati et al. (2010) – SuRFNCD-2007[364]	25 – 64 years	Optimal HOMA-IR cut-off for diagnosis of metabolic syndrome (IDF and ATPIII) = 1.775	Lower than current study
Do et al. (2010)[381]	Non-diabetic hospital personnel Thailand BMI <25 kg/m^2	50th percentile = 0.9 75th percentile = 1.4 90th percentile = 1.6	Lower than current study
Tomè et al. (2010)[370]	18 – 104 years Diabetic and non-diabetic	50th percentile = 1.7	Lower than current study
Gayoso - Diz et al. [EPIRCE study (2011)][351]	20 – 92 years Spain Non-diabetic	50th percentile = 1.7 66th percentile = 2.2 75th percentile = 2.5 80th percentile = 2.7 90th percentile = 3.5	Similar to 75th and 80th percentiles
Simarro et al. (2011)[382]	General population in Spain >18 years of age	90th percentile = 1.24	Lower than current study

**BMI = body mass index; HOMA-IR = homeostasis model assessment – insulin resistance; NIDDM = non-insulin dependent diabetes mellitus; IDF = International Diabetes Federation; ATP = Adult Treatment Panel*

The QUICKI was performed in this study as a confirmatory measurement to the HOMA-IR because it has also been relatively well validated with the HEC method in a vast array of populations, such as non-obese, obese and type 2 diabetic patients.[20,218,226,229,232,233] Katz et al. has credited the QUICKI with a greater predictive value of IR than the HOMA-IR, which has been said to perform better than its predecessor when compared with the HEC and minimal model.[20] It has been mentioned that it is often useful to make use of more than one IR index in a study to corroborate findings and to prevent incorrect conclusions from being drawn.[194]

As with the HOMA-IR, there is no consensus regarding a standardised QUICKI cut-off point. This would seem logical since both of these fasting indices rely on the same underlying physiological standard[13] and appear to be similar when comparing all aspects.[196] Whilst the increasing values of HOMA-IR indicate IR, decreased values of QUICKI are indicative of an IR state. Proposed cut-offs for IR have also been suggested for the QUICKI and are shown below in Table 4.2.

Table 4.2: Proposed QUICKI cut-off points

Source	Population/rationale	QUICKI cut-off value
Katz et al.[20]	Mean values: • Non-obese • Obese • Diabetic	 • 0.382 • 0.331 • 0.304
Ascaso et al. (2003)[295]	Healthy subjects (hospital personnel)	0.33
Gokcel et al.[379]	Healthy subjects	0.347
Hrebicek et al.[235]	Lower limit of 95% confidence level in healthy individuals (in which metabolic syndrome may typically manifest)	0.357
Pathcare Laboratories (South Africa)[383]	Accompanied by relevant clinical markers of metabolic syndrome	<0.357
Lancet Laboratories (South Africa)[377]	• Non-obese • Obese	• <0.375 • <0.321

QUICKI = quantitative insulin sensitivity check index

This equates to a range for QUICKI cut-offs of between 0.304 and 0.382, into which the calculated QUICKI for this particular study (0.336) falls comfortably. The QUICKI cut-off identified the same participants as the HOMA-IR in this particular study, which equates to an overall IR prevalence at baseline of 25.4%.

In an attempt to improve reporting rates of IR in this study, a sub-analysis was performed by excluding the participants classified as having metabolic syndrome (IDF and ATP III criteria) from the total baseline study population. This was done because IR is often seen as being synonymous with the syndrome, and it was interesting to investigate if the inclusion of any of these participants would affect the prevalence of IR. The previously-explained principle of calculating HOMA-IR as the 75th percentile was applied with the respective participants excluded, and this had little impact on the overall IR prevalence rate at baseline (24.6% with IDF and 25.9% with ATP III). The two participants who presented with raised fasting glucose levels were excluded for interest sake in an additional sub-analysis, which produced the same HOMA-IR cut-off point as the IDF exclusions.

Since the HOMA-IR cut-offs decreased slightly with the excluded participants, this signified slightly more participants being identified with IR at two months, but the same six participants were identified with all three HOMA-IR cut-off points. This would suggest congruence among the cut-off points and relatively minimal impact on the overall prevalence of the excluded participants.

4.8 INSULIN RESISTANT vs. NON-INSULIN RESISTANT GROUP

Insulin resistance is a condition typically found in those who are overweight/obese and/or have an increased amount of visceral body fat, as well as in those individuals who suffer from a range of IR-associated conditions, such as DM, PCOS or metabolic syndrome.[14,15,150,151,314] It is, therefore, interesting to assess which factors (either demographic, anthropometrical or biochemical) were related to possible IR development in this particular TB population. This is of importance, given that the participants recruited for this study were not suffering from obesity or any other IR-related condition and did not fit the ‘typical’ profile of an IR patient.

The development of IR has often been linked to the process of aging,[20,191] since age progression is generally associated with greater gains in body weight and/or fat mass, especially in the central area of the body,[314] as well as with an increased prevalence of chronic diseases of the lifestyle, including metabolic syndrome.[147,384] As previously mentioned, the presence of abdominal adipose tissue is linked to both hyperinsulinaemia and IR.[314] In this study population, there was a significant difference in age between the IR and non-IR groups, with the younger patients displaying a greater prevalence of IR. It is, however, important to note that the majority of participants (54.2%) in the study fell into the youngest age group (18 - 30 years).This is corroborated by the biochemical results in the present study in which younger participants showed a higher fasting insulin measurement. The systematic review conducted by the Jeon et al. group in 2008 also documented a more prominent relationship

between DM and TB among the younger group[269] although this may be due to the type of diabetes concerned (type 1 vs. type 2).

A study by Esteghamati et al. found that HOMA-IR levels underwent a slight decrease after the age of 50 years, signifying a lower prevalence of IR in this older age group,[365] whilst a study performed by Simarro et al. in 2011 found an increasing trend with age, except for subjects older than 65 years in which IR levels decreased.[382] In another study (EGIR in healthy Europeans), age was not deemed to be a significant cause of IR,[320] whilst the EPIRCE study was in agreement with the current study by showing slightly decreasing HOMA-IR levels with aging.[351] This could signal the need for increased awareness of greater disease potential among the more youthful TB population, despite contrasting results to date.

Another theory that has come to the fore involves the evolutionary debate encompassing the 'thrifty hypothesis'.[385] This hypothesis was generated to provide some explanation, however speculatory, regarding the escalation of global obesity rates and its postulated relationship with the tuberculosis epidemic seen much earlier in history. This hypothesis postulates that persons who had sufficient body fat stores were more likely to survive famine-like conditions and starvation (or TB) and, thereby had an immediate advantage over their leaner counterparts in the short term. Whilst this may be plausible, the undesirable side-effects of this thrifty hypothesis would lead to unfavourable consequences such as the development of chronic conditions (i.e. metabolic syndrome) during times when dietary intake was plentiful and starvation was not commonplace.[386,387] This could apply in South Africa because although largely still a developing country, many geographical areas do not suffer from food insecurity such as the Western Cape, which was recently shown to have the lowest hunger rate (16.4%) in the country.[301] This could subsequently trigger excessive dietary intake, leading to the rise in obesity and non-communicable disease rates. It could also be postulated that the specific fat content in the body could act as a 'set point' for triggering the metabolic and immune responses (or IR) whereby each individual varies.[14,387-389] This, however, only provides grounds for speculation at present.

There is generally a greater risk of IR with increasing anthropometrical measurements,[382] and positive correlations have been documented between IR and BMI,[367,390] waist and hip circumference, body fat content and weight gain.[168,214,351] There was a significant difference between the IR and non-IR groups at baseline with regard to the interpretations of percentage body fat, subscapular skinfold and the sum of subscapular and triceps skinfolds. In addition, measurements of BMI and fat mass (not documented in Chapter 3) were all slightly higher in the IR group (although not significantly). This could perhaps be indicative of the phenomenon of increasing anthropometric measurements with IR, although this should be repeated in future studies.

A study conducted in 2012 by Addo et al. amongst adolescents in the United States showed that skinfolds (tricep and subscapular) were able to identify individuals at risk of developing IR.[391] The study also showed that amongst the male study population, the subscapular skinfold had a stronger association with HOMA-IR than with the tricep measurement, suggesting the greater likelihood of developing IR with increased central adiposity compared to peripheral distribution.[391]

In the current study, only the interpretation of subscapular skinfold and sum of subscapular and tricep skinfolds showed a significant difference between the IR and non-IR groups. The mean skinfold values were higher among the IR group (although not significantly so), which would seem to agree with the Addo et al. study. However, results of a multiple regression analysis showed that IR can be explained by waist circumference, sum of skinfolds and fat mass (although only yielding a 20.4% variation). Individual skinfolds (subscapular or tricep) did not contribute to IR at all. The sum of skinfolds could therefore perhaps be used as a marker to identify those at risk of developing IR, given the link between increased subcutaneous fat and IR. Although no significant differences were seen in this study regarding the waist circumference classification, an increased waist circumference measurement, largely identifying individuals at risk of excess VAT, has long since been associated with IR.[12,15,153,392] The greater the amount of intra-abdominal fat present, the higher the risk for the development of glucose intolerance[393] and the greater severity of IR.[394] This easily-performed measure of central adiposity has also been linked to the chronic, often low-grade inflammation commonly found in patients with IR.[17,156] Although it is possible for IR to develop in individuals presenting with a normal body weight, as seen in the current study population, IR is more commonly associated with obesity, be it visceral or total BMI.[12,15,153] Obese patients are more likely to present with the majority of their fat tissue as visceral fat, which by nature is more pro-inflammatory, whilst non-obese individuals (as was seen in the current study) store a greater proportion of their fat as subcutaneous tissue, consisting of less pro-inflammatory markers.[388,395,396]

An increased waist circumference would thus seem to indicate a greater likelihood of developing, or having, active IR because it may result in the excessive secretion of inflammatory components such as TNF-α, IL-6, MCP-1 and resistin.[166] Visceral adipose tissue, or adipose tissue in general, has been theorised to play host to a vast number of mycobacteria, thus potentiating the risk for active disease development.[267] Insulin resistance pathogenesis typically occurs as a result of free fatty acid release from the visceral tissue, resulting in an influx of various hormones and cytokines, giving rise to an inflammatory response.[164] In addition, excessive amounts MCP-1 (an adipokine) have specifically been linked to the development of IR, largely through the inhibitory action on insulin.[267] Although there were no significant differences in waist circumference classification seen in this study, an increased waist circumference, apart from increasing IR prevalence, has also been linked to a

concomitant reduction in HDL-cholesterol,[397] another risk factor for the development of metabolic syndrome.

Because IR is largely termed an 'inflammatory' condition, levels of CRP (a positive acute phase protein) are often raised,[144] although not as much as in a more acute condition. The inflammation typically found in IR is more of a 'low-grade' inflammatory state, which is often the result of the production of cytokines from VAT.[398] The CRP levels are often produced as a response to the release of various cytokines designed to enable the inflammatory response.[399] A similar reaction is seen with the concomitant positive association between IR and the metabolic syndrome, and other markers of the acute phase response.[17,400-403]

One might thus have expected the CRP levels of the IR participants to be higher than the non-IR group, given the inflammatory nature of IR itself, its relationship with the APR and the fact that TB patients generally have increased CRP levels. However, the converse was seen. Given the fact that patients included in this study did not fit the conventional 'metabolic syndrome' or obesity profile, it could be speculated that they may have slightly higher levels of subcutaneous fat, compared with visceral tissue, which would, therefore, render a slightly less inflammatory profile, and this could have an inhibitory effect on CRP levels in the blood. Although the IR group seemed to display a lesser inflammatory response when compared with the non-IR group, the sample size was relatively small, and this finding should definitely be repeated in future studies.

The chronic inflammation often seen in IR and metabolic syndrome has been postulated to be due to the following factors: (1) IR may be set-off by the chronic inflammation seen in obesity; (2) the metabolic syndrome is strongly associated with increased cardiovascular risk, and CRP may, therefore, be increased due to a pre-existing atherosclerotic state; (3) reduced insulin sensitivity may be linked to an enhanced expression of CRP; and (4) there is a strong association between chronic inflammation and body fat content.[17]

Literature has also documented the correlation between IR occurrence and biochemical measures, namely raised triglycerides, reduced HDL-cholesterol, increased glucose and insulin, as well as hepatic enzymes.[169] In a large study conducted in Spain (EPIRCE), HOMA-IR levels showed a non-linear association with blood pressure (systolic), HDL-cholesterol and triglycerides.[351] Other factors shown to correlate well with IR include blood pressure (although hypertension is strongly associated with obesity),[214] as well as a family history of diabetes, [171-174] although the latter was not taken into account in the present study.

Although there was no significance regarding any of the metabolic syndrome markers between the IR and non-IR groups, the IR participants displayed slightly higher levels of blood pressure (both systolic and diastolic), fasting glucose and triglycerides. Despite there being no significant differences between the classifications of HDL-cholesterol and waist circumference between the groups, this may indicate IR being a pre-cursor for the development of certain clinical conditions associated with metabolic syndrome and could highlight the need for more preventative action prior to development of the syndrome.

Although the above discussion primarily concerns the IR and non-IR groups at baseline, it was observed that the IR participants at the follow-up periods displayed a similar trend with regard to the behaviour of the variables (both anthropometric and biochemical). There were also no differences between the sputum conversion rates (AFB) in the IR and non-IR groups in this study, despite all of the IR participants having converted by the two-month mark. Considering the evidence available for sputum conversion among diabetic patients (which encompasses the condition of IR), the results are inconclusive,[243] although some studies have found a longer conversion time in DM.[264,404-406] It is unclear whether diabetes has an advantageous or disadvantageous effect on the smear results.

Therefore, the objectives entailing the assessment of differences between IR and non-IR participants as well as demonstrating no relationship between IR status and sputum results at any of the measured time frames were also achieved.

4.9 SPUTUM RESULTS

The majority of participants in the current study presented with a smear-positive sputum result at baseline, which indicated a more ‘severe’ or infectious form of TB compared with their smear-negative counterparts. Although there were no significant differences between any of the anthropometrical and biochemical markers (excepting fasting glucose), one could perhaps have expected the smear-positive group to have presented with slightly more derangements of these parameters given their propensity to be more ‘unwell’ than those in the negative group. It is important to note that the patients recruited for this study, although presenting with infectious TB, were still relatively well and able to attend the health facility, and as such, disease severity may not have such a prominent effect when compared with a hospital-type setting.

Literature has reported the relationship between a decreased BMI and the increased radiological extent of TB (i.e. degree of sputum positivity).[407] Given the supposed bi-directional relationship

between TB and malnutrition, it might be speculated that the more severe form of TB would result in a greater degree of weight loss (albeit both fat and lean body mass) and ultimately, an impaired nutritional status. Although there was no significance reported, the smear-positive group did present with lower mean values for weight, BMI, waist circumference, sum of skinfolds, fat mass, fat free mass and percentage body fat. This lack of significance could perhaps be caused by the small sample size because groups were not stratified into statistically representative groups according to sputum results.

Similarly, regarding the biochemical values, the only variable to show a significant difference between the positive- and negative-smear groups was the fasting glucose, which was lower in the sputum-positive group. This is an unexpected finding because it may be postulated that the smear-positive group was subjected to a greater form of inflammation than the negative patients, and this heightened inflammatory response may have given rise to greater IR, which may lead to a reduction in insulin production and a concomitant hyperglycaemic response.[244] Diabetes also seems to have a strong causative link to the more infectious type of TB,[261] as well as a greater prevalence in the smear-positive group in this study. Although fasting insulin was lower in the positive group (although not significantly so), there was no sign of hyperglycaemia in these patients, which is a converse finding and definitely warrants further investigation. Due to the decreased mean fasting glucose value, this resulted in a lower HOMA-IR and higher QUICKI value in the positive group, which correlates with the converse finding of a seemingly decreased IR status in the more 'unwell' patients.

The finding of raised CRP levels in TB patients was documented in Chapter 4.3: Biochemistry Results. Literature has also reported significantly higher levels of CRP among smear-positive patients,[107,404] given the nature of CRP to act as an indicator of inflammation intensity. Since the smear-positive patients present with a more severe and radiologically advanced disease, they generally have an increased inflammatory load,[113] which often leads to a poorer prognosis.[408] A higher CRP level has also been linked with the severity of lung dysfunction.[126] There seemed to be higher mean CRP levels and lower albumin levels in the smear-positive group in this study and although not significant, could perhaps be in agreement with the above literature.

Differences in lipid profile (namely total cholesterol, HDL-cholesterol and triglycerides) have been documented by researchers, who stated that lower levels of these markers were found in patients with more advanced stages of infection.[114] This is again explained by the increased inflammatory response seen in patients with the more severe form of the disease.[114] Although there was no significance, results generated by this study showed a lower mean value for total and HDL-cholesterol, but a higher mean triglyceride level, and this should also be followed up.

The secondary objective of assessing changes between smear-positive and smear-negative participants was also achieved, and although minimal significant differences were seen, these could be pursued in subsequent studies.

One of the main reasons why participants in the current study were chosen to be followed up at two and five months was to allow for the coinciding with fixed sputum follow-up dates as implemented by the South African DoH. Participants had their sputum re-tested at both periods if they presented with a positive or unclear sputum at baseline. The anticipated sputum conversion is of vital importance to allow for assessment of treatment efficacy because a smear that remains positive at the five-month mark would indicate a treatment failure.[5] Researchers have suggested possible causative factors of treatment failure, which include a higher prevalence of localised disease with chest radiography, little radiological improvement over time, greater drug resistance and high default rates.[409]

Those individuals who are able to convert from smear-positive to smear-negative within a certain period of time are deemed ‘fast’ responders, whilst those that present with a persistent positive smear are termed ‘slow’ responders.[410] Smear results and their subsequent conversion are often a very useful tool in determining a patient’s response to TB treatment.[316] The WHO is currently aiming for a treatment success rate of a minimum of 85% of cases, which will hopefully bring South Africa closer to achieving the MDGs or SDGs, in terms of lowering TB incidence and mortality rates.[24]

The current study population showed promising signs of sputum conversion because the majority of smear-positive patients at baseline had converted by two months (the intensive phase), and all but one participant had converted at the five-month mark, thus indicative of treatment success and well above the WHO cut-off of 85%. However, one of the patients in the follow-up group who had sputum converted relapsed a few months after treatment completion and was placed on a new course of therapy. This indicates the need for at-risk patients to be followed-up even after treatment completion due to confounding relapse risks such as poor nutritional status, default history and known household contact with TB suspects. This may, however, be somewhat of a challenge given the overburdening, understaffing and resource depletion of the public health system in South Africa.

Because significant differences were seen in sputum TB results between baseline and two months, as well as baseline and five months, the secondary objective of assessing these changes was also reached.

4.10 STUDY STRENGTHS AND LIMITATIONS

This study was the first to investigate the prevalence of IR in a population of adult patients newly diagnosed with PTB, by means of the HOMA-IR and QUICKI indices. Many studies have explored the phenomena of increased rates of diabetes occurring in TB patients, but this study exclusively considered IR status and excluded all previously diagnosed diabetic patients. In addition to the novel data presented, the study was further strengthened by the principal researcher performing all administrative, anthropometrical and transport functions, thereby reducing the risk of inter-observer variability or bias. Only one laboratory was used for the analysis of biochemical measures, which allowed for comparison between samples in this particular study, especially regarding the evaluation of the fasting insulin marker.

The HOMA-IR and QUICKI, despite their shortcomings, are two of the most prominent fasting indices used in clinical research and have shown good correlation with the gold standard method of testing and diagnosing IR. Because these indices have been linked to defining hepatic IR, which often occurs before peripheral IR sets in, it may be possible to intervene in a timely manner with patients presenting with early onset IR. The analytical component of the study added strength to the baseline component since participants were followed-up over time, and inferences were made from generated data. The follow-up period also extended beyond the two-month intensive phase, in which the majority of significant results were seen. In addition to these strengths, the researcher was also able to achieve an excellent recruitment rate (98.3% of the target study population). Patients were enrolled in the study shortly after treatment commencement, thus reducing the confounding effect of the TB medication on the IR status. A subgroup analysis was also performed to re-evaluate the HOMA-IR and QUICKI cut-off points if patients were found to have any pre-disposing IR conditions post-study recruitment, thus reducing bias.

Due to the novel nature of the study, a few limitations arose that were largely beyond the control of the researcher.

The nature of the HOMA-IR and QUICKI indices is such that it makes inter-study comparison very challenging, given the paucity of standardised reference values, the reliability in the quality of glucose and insulin measurements, the lower accuracy in normal or near-normal weight individuals and the lesser relevance in certain ethnic groups. The fasting insulin measurement also presents with countless problems, given its short half-life, pulsatile action and variability between assays. Although it is recommended to perform three insulin measurements, many studies have used one basal insulin measurement, as was done in this study. It has also been reported that IR is often higher in the morning due to the combined action of cortisol and FFA.[6] This unfortunately co-incided with the time

of data collection, although this was beyond the researcher's control given logistical issues with phlebotomy samples and patient compliance with fasting requirements.

In addition, there is currently no consensus as to which metabolic syndrome guidelines (IDF, ATP III, WHO, etc.) should be used because this is an ever-changing concept. Furthermore, it was unclear if patients presented with hyperglycaemia before recruitment into the study since the researcher recruited individuals purely on the absence of any pre-diagnosis diabetic conditions in the medical folder at the facility. In the patients who presented with hyperglycaemia, this could be due to previously undiagnosed DM, newly diagnosed IR or to transient hyperglycaemia, often found in TB patients. Due to logistical and ethical issues, the researcher was also only able to access the patients once they had started treatment and, therefore, all patients seen were receiving anti-TB medication. Given the complexities in working with human subjects, exact compliance with the TB medication and fasting on the day of sample collection were reliant upon the patient's confirmation thereof.

Other limitations included inherent facets of the study design, such as the cross-sectional nature of the baseline aspect of the study, which resulted in no conclusions of causality being drawn. As the study was primarily planned and executed by the principal researcher, logistics only allowed one study site to be used for recruitment, which could impact on the generalisability of results. Although not a primary objective of the study because no stratification was done, caution needed to be applied in the assessment of the demographic findings because this could limit the reliability of any associations drawn from a heterogeneous study population. In closing, the groups that were compared in the sub-analyses (IR vs. non-IR; sputum-positive vs. sputum-negative) were not known before data analysis was completed, and these groups were not always of an equal sample size, although this was taken into account with appropriate statistical tests.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This study aimed to assess if there was an association between TB and the development of IR in newly diagnosed ambulatory adults between the ages of 18 and 65 years with PTB in the Cape Town area of South Africa. This was measured via an assortment of methods, and certain patients were followed-up whilst on TB treatment. The current study is, to the knowledge of the author, the first to assess IR prevalence using the HOMA-IR and QUICKI indices in a population of PTB patients. The results generated from this study show an association between TB and the development of IR and fulfill the overarching aim of investigating this relationship, shedding light on this novel research avenue, providing scope for possible future research ventures.

A number of important findings were made and are outlined below in accordance with the objectives of the study. These findings, as well as recommendations for future research and clinical practice, will hopefully contribute to the available knowledge of the subject at hand.

5.1.1 To Assess if IR was Present in Participants with PTB via the Testing of Anthropometrical and Biochemical Measures, as well as Diagnostic IR Tests

- Using the calculated cut-off point for HOMA-IR (2.477), the IR prevalence at baseline was 25.4%, which equates to 1 in 4 persons with newly diagnosed PTB having IR. These findings were echoed by the QUICKI.
- This prevalence rate falls within the proposed range of 20 - 40% IR in the general population, of whom the majority are healthy persons (i.e. do not have TB).[145,146] The rates among this study population are, therefore, found to be quite high.
- The metabolic syndrome criteria used (IDF and ATP III) failed to classify the majority of patients who presented with IR according to HOMA-IR and QUICKI calculated cut-offs.
- Adjusting for patients with the metabolic syndrome according to both IDF and ATP III criteria had little effect on the total IR prevalence at baseline.

5.1.2 To Assess Changes in Anthropometrics and Body Composition at Baseline and Follow-up Periods

- The majority of participants in the study had a normal BMI. However, some were classified as overweight (BMI>25 kg/m^2). Being underweight (BMI<18.5 kg/m^2) is often viewed as a risk factor in the development of TB disease, and the prevalence of normal or over-nourished patients in this study might be due to the significant overweight/obesity epidemic that South Africa is currently facing.

- It is, however, pertinent that the majority of studies evaluating the nutritional status of TB patients at baseline have found these persons to be more underweight than their healthy counterparts. Many of these studies, however, were conducted among hospitalised patients, perhaps for ease of access to participant data. The current study investigated facility-based ambulatory patients, which could also signify a less severe or less undernourished patient population, linking to the largely 'normal' nutritional status mentioned above.
- Despite the majority of patients having a BMI within the normal range, the total body fat was still relatively low, and most patients were classified as being either 'underweight' or 'slim' when assessed. The phenomenon of fat mass loss (as well as lean body mass loss) has been well documented in literature, but should be monitored, especially given the significant gains in fat mass commonly seen whilst on treatment that could later prove to be detrimental.
- The majority of anthropometrical measurements showed a significant increase whilst the patients were on treatment, with the greatest changes taking place during the intensive phase (first two months of treatment). However, some patients failed to gain more than 5% weight over the five-month period and/or remained in the underweight BMI category, thereby placing them at greater risk of treatment failure and/or relapse at a later stage.
- There was a significant increase in both fat and fat free mass over time. Whilst the increase in fat mass has been previously described, the increase in fat free mass is encouraging, given its association with improved functionality, quality of life and longevity.
- The increase in fat mass seemed to be associated with gains in both the peripheral and trunk area (albeit slightly more in the trunk area).

5.1.3 To Assess Changes in Biochemistry and Blood Pressure at Baseline and Follow-up Periods

- The most noticeable derangements in baseline biochemical values showed the majority of patients having an increased CRP level and a decreased HDL-cholesterol level, both of which could be indicative of the inflammatory state noted in TB patients.
- There were relatively few presentations of hyperglycaemia and/or abnormal insulin levels in the study although IR rates were found to be high. This raises the question as to what caused the IR in these patients. Possibilities include either chronic infection or medication although this concept needs to be explored further.
- With regard to the changes in biochemical markers over time, the CRP, albumin and white cell count all showed an improvement (especially in the intensive treatment phase), which would seem to indicate an improvement in the inflammatory state or infectious state of the body. Although the cholesterol levels (total, LDL and HDL) increased in the first two months

of treatment, indicating a possible improved response to infection, there were no differences noted over the last few months, which is an interesting concept to revisit.

5.1.4 To Assess Changes in IR Status at Baseline and Follow-up Periods

The HOMA-IR and QUICKI levels also improved with time, although not significantly, which may indicate a seeming reduction in IR. Some patients who had IR at baseline presented with normal levels at two months but then redeveloped IR at five months. In addition, some patients were non-IR at baseline, but developed it at two and five months. These two occurrences possibly question the stress hyperglycaemia or reduction in inflammation theories presented in the literature.

5.1.5 To Assess Changes in Sputum TB Results at Baseline and Follow-up Periods

There were significant differences in sputum results between baseline and two months, as well as baseline and five months. This is indicative of the majority of patients having converted to sputum smear-negative by the five-month mark, signifying an improved clinical state.

5.1.6 To Determine the Relationship between IR Status and Sputum TB results at Baseline and Follow-up Periods

No significant differences were found in this category.

5.1.7 To Assess Differences between IR and non-IR Participants

- Differences noted in IR patients compared with their non-IR counterparts included the following: IR patients were younger, had a significantly higher interpretation of skinfolds and percentage body fat (signifying a ‘greater’ fat mass) and higher fasting insulin values. Although no other significant results were noted, IR patients generally showed a pattern with markers of the metabolic syndrome (increased fasting glucose, triglycerides and blood pressure).
- Mean CRP levels were lower in patients with IR compared with their non-IR counterparts although not significantly so.

5.1.8 To Assess Differences between Sputum-Negative and-Positive Participants

When comparing differences between the sputum smear-positive and negative groups, only the fasting glucose measurement was significant; the sputum-positive group had a lower fasting glucose level than the negative group and ultimately 'less' IR according to HOMA-IR and QUICKI.

The diagnosis of TB has previously been linked to consumption of harmful substances (nicotine and alcohol), as well as a low BMI, poorer educational level, unemployment and inferior household wealth.[411] If one considers these factors, it can be seen that all either affect or are affected by nutrition at some point, be it lowering dietary intake or influencing access to food, or in the case of a low BMI, rendering the immune system defenseless against infection. This, therefore, equates to a 'vicious cycle', which could perhaps be broken through the preventative provision of appropriate nutritional care and far-reaching education. Similarly, nutrition therapy induced action could also be utilised in the prevention and management of IR in TB patients, via dietary adaptations and physical activity, before the disease manifests.

If one considers the dire TB statistics facing South Africa at the current time, which include the country being labelled as having the highest incidence and prevalence rates and having a large proportion of multi-drug resistant TB and TB-HIV co-infected cases,[48] there is definitely a need for action in this arena. The compounding factor of diabetes, given the high level of non-communicable diseases in South Africa, is also reason for concern because diabetes has been found to increase the rate of mortality and treatment failure, as well as the relapse rate in those with TB.[280] Appropriate preventative screening methods, glucose control and monitoring of patients presenting with diabetes, impaired glucose tolerance or IR should be implemented at all stages of treatment. If patients are found to have IR, these individuals should be earmarked as at-risk patients, given the propensity of IR to result in chronic illnesses such as cardiovascular disease and diabetes. It would, therefore, be beneficial to identify these individuals at an early stage to prevent these probable complications from occurring.

Given the current nutrition transition experienced in South Africa, coupled with crippling rates of both communicable and non-communicable diseases, this will need to be executed within a multi-disciplinary framework that is adequately equipped to address all facets of the problem at hand.

5.2 RECOMMENDATIONS

5.2.1 Clinical Practice

- Integrative bi-directional screening (i.e. screening for DM in newly diagnosed TB patients and screening for TB in diabetic patients) should ideally be implemented in health facilities nationwide, which will address both communicable and non-communicable forms of disease.
- Because screening for DM in active TB patients is generally a more cost-effective alternative, this should be done with a simple measurement (such as a fasting or random blood glucose measurement) that does not infringe on the vast workload of staff members. Although testing of baseline urinary glucose and ketones in all TB patients is currently part of the national DoH TB management guidelines, ideally blood glucose should also be measured in all patients, not only in those who display symptoms. Those patients who are found to have increased glycaemic values should be retested soon after by means of a confirmatory method such as an OGTT or HbA1C, of which the latter is probably more practical. Screening should also encompass an enquiry into the patient's family history of DM or other hyperglycaemia-related conditions or symptoms.
- Patients presenting with hyperglycaemia should be monitored throughout the treatment period in order to dispel the transient hyperglycaemia effects or the side-effects of medication before actively being diagnosed with DM. Despite normalisation of glucose levels, the previously increased glucose levels may still pose a future risk for an individual.
- Patients found to be more susceptible (retreatments or previous defaulters, known diabetics, newly diagnosed IR/DM patients, patients with impaired nutritional status, HIV-TB co-infected patients, etc.) should be referred for appropriate nutritional intervention, either via dietary counselling or supplementation.
- Patients with undesirable markers (such as low BMI, inadequate weight gain, raised CRP levels, lack of sputum conversion, poor clinical presentation, etc.) should be continually assessed in terms of nutritional status, regardless of whether they have successfully completed their course of treatment or not.
- Patients identified with IR should be closely monitored and appropriate counselling given to prevent the onset of any of the conditions associated with the metabolic syndrome. One could also monitor and evaluate an excessive waist circumference with regard to increasing IR risk or inflammatory profile.
- Since the intensive phase of treatment seemed to yield the most significant changes, it would be prudent to interact with the patient at an early stage in order to improve the outcome and lessen the negative effects of TB disease. Patients with possible IR and those with an

impaired nutritional status upon commencement of treatment could be counselled in terms of dietary improvements and physical activity levels.

- Although a recent Cochrane review[86] could not find sufficient evidence to support the blanket issuing of nutritional interventions for undernourished TB patients, one must not discount this option entirely. The Nutritional Therapeutic Programme of the DoH should, therefore, be correctly implemented in combination with adequate dietary monitoring by a nutritional professional such as a registered dietician or nutritionist, or in their absence, a registered nurse.
- Physical activity (as tolerated) should also be emphasised, in conjunction with support from a suitable health care professional in the multi-disciplinary team (such as a physiotherapist, biokineticist or occupational therapist). Increased physical activity will be beneficial to all TB patients because it is able to reduce total and visceral fat and build lean body mass, which is crucial. Increased activity also has a diminishing effect on the IR status in the body.
- Constant re-evaluation of strategies to prevent high default rates should be carried out, especially in patients who never begin treatment despite a positive diagnostic TB test, although this remains challenging.

5.2.2 Future Research

- Given the high prevalence of IR in this particular study population, it may prove prudent to investigate the prevalence of IR in other TB-specific patient populations. Because the current study focused largely on the uncomplicated, drug-susceptible form of TB, assessing IR status in multi-drug resistant (MDR) and extensively drug-resistant (XDR) patients might yield interesting results.
- One could also perhaps target vulnerable TB patient populations, such as miners, prisoners, children or the elderly to determine if they display a greater propensity for IR compared with the current population. The children/adolescent population might be extremely valuable to assess, given the current trend of earlier development of previously adult-onset diseases such as diabetes and cardiovascular disease.
- It may also be of value to include a comparison of HIV-negative with co-infected TB patients to assess the impact of the ‘double burdened’ patients on the IR status, since HIV alone has previously been linked to development of IR.
- A greater number of recruitment sites could be introduced in future studies. This would allow for a faster data-collection process, as well as for inter-site comparisons to be made in terms of demographic profile (providing the sample size was sufficient).
- Although the current sample size was adequate, a larger sample size would perhaps corroborate findings shown in the current study.

- It would be prudent to re-assess the patients who displayed IR at the five-month period after completion of their TB treatment. They could be investigated for worsening IR status or possible development of the metabolic syndrome. Due to time limitations and logistical issues, this was unfortunately not part of the scope of the current study.
- Additional tools could be utilised in the assessment of IR status (i.e. HEC method) as a comparison with the results found in this study.
- An intervention study could possibly be conducted at a later stage that could assess the impact of dietary and lifestyle counselling on IR progression in TB patients. Alternatively, different forms of TB (drug susceptible, MDR, XDR, HIV co-infected, etc.) could be compared with one another to assess IR severity.
- Although it was not possible in the current study, a desired outcome of a future study could be to determine a specific biomarker cut-off (e.g. CRP) that would be able to identify IR in TB patients and largely act as a diagnostic tool.

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ADDENDA

ADDENDUM A

Additional metabolic measurements for metabolic syndrome research devised by the IDF[9]

Abnormal body fat distribution	• General body fat distribution (DEXA) • Central fat distribution (CT/MRI) • Adipose tissue biomarkers: leptin, adiponectin • Liver fat content
Atherogenic dyslipidaemia (beyond elevated triglycerides and low HDL)	• ApoB (or non-HDL-cholesterol) • Small Low Density Lipoprotein (LDL) particles
Dysglycaemia	• Oral Glucose Tolerance Test (OGTT)
IR (other than elevated fasting glucose)	• **Fasting insulin**/pro-insulin levels • **Homeostasis Model Assessment-IR (HOMA-IR)** • IR by Bergman Minimal Model • Elevated free fatty acids (fasting and during OGTT) • M value from clamp
Vascular dysregulation (beyond elevated blood pressure)	• Measurement of endothelial dysfunction • Microalbuminuria
Pro-inflammatory state	• Elevated high sensitivity **C-reactive protein** • Elevated inflammatory cytokines (e.g. TNF-α, IL-6) • Decrease in adiponectin plasma levels
Pro-thrombic state	• Fibrinolytic factors (PAI-1, etc.) • Clotting factors (fibrinogen, etc.)
Hormonal factors	• Pituitary-adrenal axis

HDL = high density lipoprotein; IR = insulin resistance; DEXA = dual energy x-ray absorptiometry; CT = computerised tomography; MRI = magnetic resonance imaging; ApoB = apolipoprotein B; LDL = low density lipoprotein; TNF-α = tumour necrosis factor- α; IL-6 = interleukin-6; PAI-1 = plasminogen activator inhibitor-1

Factors highlighted in bold indicate those tested for in the current research study

ADDENDUM B

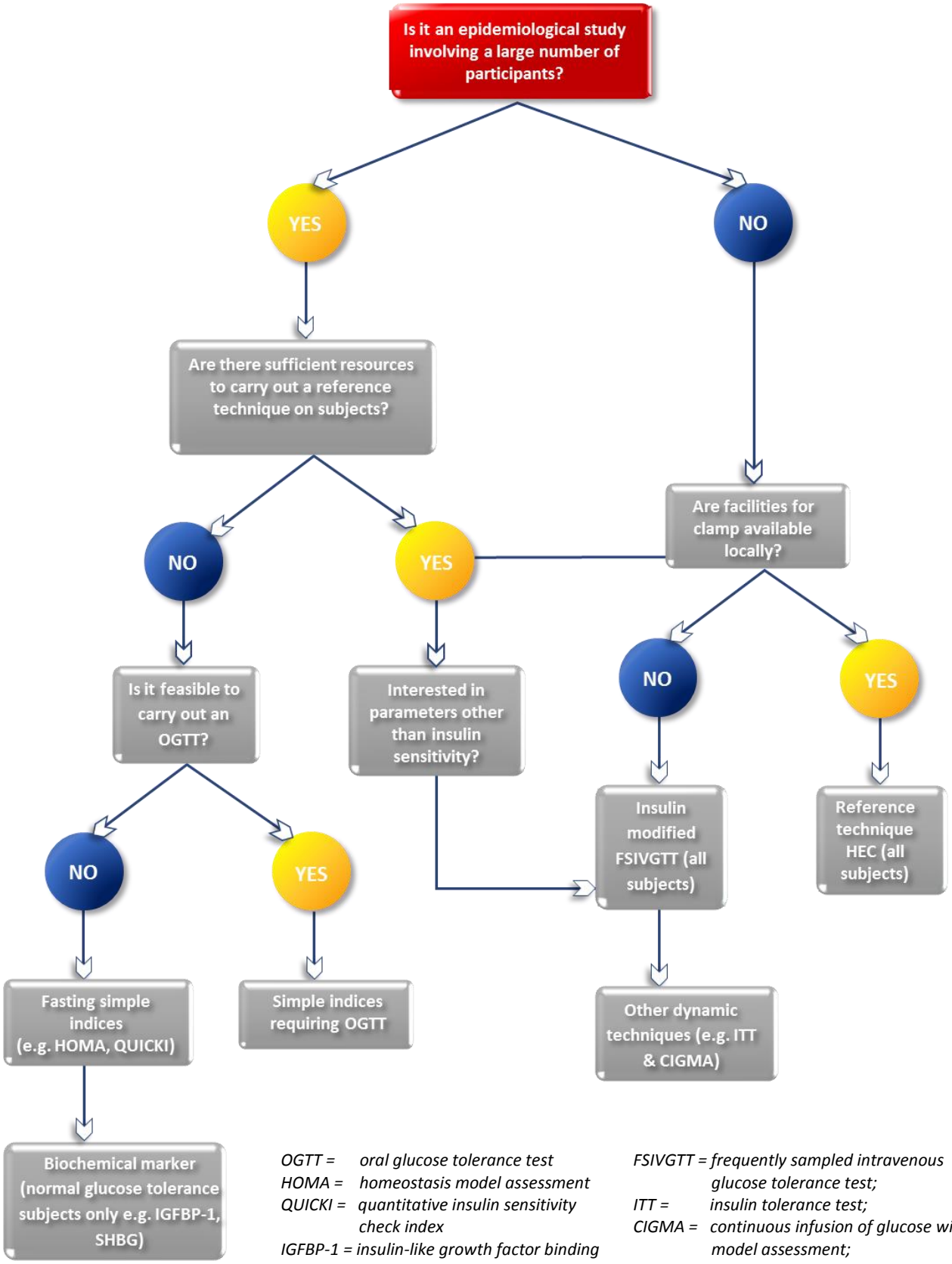
Oral glucose tolerance test derived indices[13]

Index	Formula
Avignon Index[204]	{0.137 x [10^8 / (fasting insulin$_{\mu IU/mL}$ x fasting glucose$_{mg/dL}$ x VD)]) + [10^8 / (120 min insulin$_{\mu IU/mL}$ x 120 min glucose$_{mg/dL}$ x VD)]} /2
Belfiore Index[205]	2/ [(INSp$_{\mu IU/mL}$) x (GLYp $_{mg/dL}$) +1] INSp = AUC of insulin $_{(\mu IU/mL)}$ during OGTT divided by mean values of non-diabetic subjects as a unit, and GLYp =AUC of glucose $_{(mg/dL)}$ during OGTT divided by mean values of non-diabetic subjects as a unit
Cederholm Index[412]	[75 000 + (fasting glucose – 120 minutes glucose$_{mmol/L}$) x 1.15 x 180 x 0.19 x body weight$_{kg}$] / [120 x (log mean insulin$_{\mu IU/mL}$) x mean glucose$_{mmol/L}$]
Gutt Index[413]	[{75 000 + (fasting glucose – 120 minutes glucose$_{mmol/L}$) x 0.19 x body weight$_{kg}$}} / 120 / [fasting glucose + 2 h glucose$_{mg/dL}$] / 2] / log [fasting insulin + 120 min insulin$_{\mu IU/mL}$]/2]
Matsuda Index[145]	10^4 / (fasting glucose$_{mg/dL}$ x fasting insulin$_{\mu IU/mL}$ x mean glucose OGTT$_{mg/dL}$ x mean insulin$_{\mu IU/mL}$)$^{0.5}$
Stumvoll Index[206]	0.0226 – 0032 X BMI$_{kg/m^2}$ – 0.0000645 x 120 min insulin$_{pmol/L}$ – 0.00375 x 90 min glucose$_{mmol/L}$
Oral Glucose Insulin Sensitivity (OGIS) Index[414]	Available online at *http://webmet.pd.cnr.it/ogis/index.php*
SI_{is}OGTT[218]	1/{ log [sum glucose$_{0+30+90+120 min (mmol/L)}$] + log [sum insulin$_{0+30+90+120 min (\mu IU/mL)}$)}

OGTT = oral glucose tolerance test; VD = volume of distribution; AUC = area under curve; INS = insulin; GLY = glucose; BMI = body mass index

ADDENDUM C

Protocol for selecting the most appropriate technique for assessment of IR prior to commencement of a study[5]



OGTT = oral glucose tolerance test
HOMA = homeostasis model assessment
QUICKI = quantitative insulin sensitivity check index
IGFBP-1 = insulin-like growth factor binding protein-1
SHBG = sex hormone binding globulin;
FSIVGTT = frequently sampled intravenous glucose tolerance test;
ITT = insulin tolerance test;
CIGMA = continuous infusion of glucose with model assessment;
HEC = hyperinsulinaemic euglycaemic clamp

ADDENDUM D

Data collection sheet (principal researcher)

Unique ID number of participant		
Participant Allocation: Baseline (1) or Follow-up group (2)	**1**	**2**

DEMOGRAPHIC INFORMATION:

AGE		YEARS		
GENDER	MALE		FEMALE	

ANTHROPOMETRY:

1. **Weight (at baseline)**

Measurement 1 (kg)	Measurement 2 (kg)	Measurement 3 (kg)	**Average (kg)**

2. **Height (at baseline)**

Measurement 1 (m)	Measurement 2 (m)	Measurement 3 (m)	**Average (m)**

3. **Waist circumference (at baseline)**

Measurement 1 (cm)	Measurement 2 (cm)	Measurement 3 (cm)	**Average (cm)**

4. **Hip circumference (at baseline)**

Measurement 1 (cm)	Measurement 2 (cm)	Measurement 3 (cm)	**Average (cm)**

5. **BMI (at baseline) –** Average weight and height variables used in calculation

kg/m^2

6. **Waist:hip ratio (at baseline) –** Average waist and hip circumference variables used in calculation

	:

7. **Elbow width and frame size (at baseline)**

mm	SMALL		MEDIUM		LARGE	

8. **Biceps skinfold (at baseline)**

Measurement 1 (mm)	Measurement 2 (mm)	Measurement 3 (mm)	**Average (mm)**

9. **Triceps skinfold (at baseline)**

Measurement 1 (mm)	Measurement 2 (mm)	Measurement 3 (mm)	**Average (mm)**

10. **Subscapular skinfold (at baseline)**

Measurement 1 (mm)	Measurement 2 (mm)	Measurement 3 (mm)	**Average (mm)**

11. **Suprailiac skinfold (at baseline)**

Measurement 1 (mm)	Measurement 2 (mm)	Measurement 3 (mm)	**Average (mm)**

NB! The following measurements are only to be completed if the participant is selected to be part of the follow-up group!

---

12. **Weight (at f/up)**

F/up	Measurement 1 (kg)	Measurement 2 (kg)	Measurement 3 (kg)	**Average (kg)**
2/12				
5/12				

13. Waist circumference (at f/up)

F/up	Measurement 1 (cm)	Measurement 2 (cm)	Measurement 3 (cm)	**Average (cm)**
2/12				
5/12				

14. Hip circumference (at f/up)

F/up	Measurement 1 (cm)	Measurement 2 (cm)	Measurement 3 (cm)	**Average (cm)**
2/12				
5/12				

15. BMI (at f/up)

2/12	kg/m^2

5/12	kg/m^2

16. Waist:hip ratio (at f/up)

2/12	**:**
5/12	**:**

17. Biceps skinfold (at f/up)

F/up	Measurement 1 (mm)	Measurement 2 (mm)	Measurement 3 (mm)	**Average (mm)**
2/12				
5/12				

18. Triceps skinfold (at f/up)

F/up	Measurement 1 (mm)	Measurement 2 (mm)	Measurement 3 (mm)	**Average (mm)**
2/12				
5/12				

19. Subscapular skinfold (at f/up)

F/up	Measurement 1 (mm)	Measurement 2 (mm)	Measurement 3 (mm)	**Average (mm)**
2/12				
5/12				

20. Suprailiac skinfold (at f/up)

F/up	Measurement 1 (mm)	Measurement 2 (mm)	Measurement 3 (mm)	**Average (mm)**
2/12				
5/12				

COMMENTS:

BLOOD PRESSURE:

Blood pressure at baseline

Blood pressure at 2 month follow-up

Blood pressure at 5 month follow-up

ADDENDUM E

Data collection sheet [National Health Laboratory Services (NHLS)]

Study Code:		*For laboratory use*

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Master of Nutrition Study
Tuberculosis and Insulin Resistance
University of Stellenbosch

NHLS Tygerberg Laboratory Request Form

NHLS Account:	ZSTU0608	NHLS Location:	TYGERBERG

Patient Information

Participant number:		DOB:	/ / 19
		Age:	years
Name:	N/A	Gender:	
Surname	N/A		

Other:

Clinical Information/Sample Information

Clinical	**Markers to be taken**
Serum albumin; CRP; Fasting insulin; Fasting glucose;	
Lipid profile; WCC	

Sample Taken	/ / 20
Time:	
Specimen	**Yellow:** Serum albumin; CRP; fasting insulin; lipid profile (4-5ml) **Purple**: WCC (4-5ml) **Grey**: Fasting glucose (1ml)

Phlebotomist:		Signature:	

Instructions

Please email results through to:
Ms Lauren Philips
laurenp@sun.ac.za

ADDENDUM F

Health Research Ethics Committee approval

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Approval Notice
New Application

25-Oct-2012
Philips, Lauren Ann
Stellenbosch, WC

Ethics Reference #: S12/08/227

Title: **The association between tuberculosis and the development of insulin resistance in adults with pulmonary tuberculosis in Delft, Cape Town, Western Cape**

Dear Ms Lauren Philips,

The New Application received on 24-Aug-2012, was reviewed by Health Research Ethics Committee 1 via Committee Review procedures on 03-Oct-2012 and has been approved.
Please note the following information about your approved research protocol:

Protocol Approval Period: **25-Oct-2012 -25-Oct-2013**

Present Committee Members:
Kinnear, Craig CJ
Soodat, Soraya S
Thomissen, Marie ME
Weber, Franklin CFS
Unger, Marianne M
Rohland, Elvira EL
Theron, Gerhardus GB
Els, Petrus PJJS
Davids, Mertrude MA
Barsdorf, Nicola

Please remember to use your **protocol number** (S12/08/227) on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review:
Please note a template of the progress report is obtainable on www.sun.ac.za/rds and should be submitted to the Committee before the year has expired.
The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.
Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Helene Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.
For standard HREC forms and documents please visit: www.sun.ac.za/rds

If you have any questions or need further assistance, please contact the HREC office at 0219389657.

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Ethics Letter

05-Apr-2013

Ethics Reference #: S12/08/227
Title: The association between tuberculosis and the development of insulin resistance in adults with pulmonary tuberculosis in the Western Sub-District of the Cape Metropole region

Dear Ms Lauren Philips,

Your letter reecived 12 March 2013 refers.

The Chairperson of the Health Research Ethics Committee approved the amended documentation in accordance with the authority given to him by the Committee.

If you have any queries or need further help, please contact the REC Office 0219389207.

Sincerely,

REC Coordinator
Mertrude Davids
Health Research Ethics Committee 2

ADDENDUM G

City of Cape Town approval

Civic Centre
12 Hertzog Boulevard
Cape Town 8001
P O Box 2815, Cape Town 8000
Ask for: Dr G H Visser
Tel: 021 400-3981
Cell: 083 280 8715
Fax: 021 421-4894
E-mail: helenevisser@capetown.gov.za
Website: http://www.capetown.gov.za
Ref:
Filename: TB/Addie: ES APPROVAL LETTER: ID NO: 10346

Ibhloko lokuNtu
12 Hertzog Boulevard
Cape Town 8001
P O Box 2815, Cape Town 8000
Calls: Dr G H Visser
Umnxeba: 021 400-3981
Cell: 083 280 8715
Faksi: 021 421-4894

Burgersentrum
Hertzog-boulevard 12
Kaapstad 8001
Posbus 2815, Kaapstad 8000
Vra vir: Dr G H Visser
Tel: 021 400-3981
Sel: 083 280 8715
Faks: 021 421-4894

CITY HEALTH — Specialised Health

2013 – 04 - 23

Re Research Request: "The association between tuberculosis and the development of insulin resistance in adults with pulmonary tuberculosis in the Western Sub-District of the Cape Metropole region"
ID NO: 10346

Dear Miss Lauren Phillips

Permission has been granted to do your research as per your protocol and to recruit clients at the following City Health clinic in Western Sub District:

Western Sub-District:	**Albow Gardens clinic**
Contact People	Mrs Monica Sifanelo (Sub District Manager)
	Tel: (021) 514 4122/ 084 630 2903
	Mrs Melissa Stanley (Head: PHC & Programmes)
	Tel: (021) 514 4124/072 329 6361

Please note the following:

1. All individual patient information obtained must be kept confidential.
2. Access to the clinics and its patients must be arranged with the relevant Managers such that normal activities are not disrupted.
3. A copy of the final report must be sent to the City Health Head Office, P O Box 2815 Cape Town 8001, within 3 months of its completion and feedback must also be given to the clinics involved.
4. Your project has been given an ID Number 10346. Please use this in any future correspondence with us.

Thank you for your co-operation and please contact me if you require any further information or assistance.

Yours sincerely

DR G H VISSER
MANAGER: SPECIALISED HEALTH

cc.
Mrs Monica Sifanelo
Ms Carmen Beukes
Ms Judy Caldwell
Dr Karen Jennings

ADDENDUM H

Request for permission letter:
Albow Gardens Clinic

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25th February 2013

Dear Madam/Sir

RE: Masters in Nutrition Research Project

I would hereby like to approach you for permission to conduct a study for Postgraduate Masters research purposes. The study is entitled *'The association between tuberculosis and the development of insulin resistance in adults with pulmonary tuberculosis in the Western Sub-District of the Cape Metropole region.'*

The aim of the proposed study is thus to determine whether there is an association between tuberculosis and the development of insulin resistance in adults with pulmonary tuberculosis in the Western Sub-District, with the aim of recording possible emerging trends and improved future care for persons with TB.

As principal investigator of the study, myself (or an appointed research assistant), will visit the Albow Gardens clinic, situated in the Western sub-district of the Cape Metropole region. This will be done with the sole purpose of recruiting patients for the study. All further measurements will not be done on site at the clinic (done at Brooklyn Chest Hospital), as not to increase the staff workload or create a shortage of space in the facility. The period of data collection is aimed to extend from June 2013 – March 2014. Various measurements will be performed on the selected participants (anthropometrics, biochemistry, etc.) under the supervision of the investigator and nursing sister of the research facility.

I would like to reassure you that during the hours of duty, I will refrain from any invasive interaction with the patients, without their informed consent. Consent will be obtained prior to the collecting of data, by means of individualised consent forms. The data will be kept anonymous and confidentiality of participants will, therefore, be maintained.

The investigator will also behave in a professional manner at all times and act in accordance with the stipulations set aside by the facility.

A transcript of the study protocol and final results can be forwarded to you, if you so wish.

If there are any further queries, please feel free to contact my study supervisors:

Mrs. Janicke Visser

- 021 938 9259 (work)
- jconrad@sun.ac.za (email)

Professor Renee Blaauw

- 021 938 9259 (work)
- rb@sun.ac.za (email)

Thank you for your favourable consideration and I look forward to hearing from you soon.

Kind regards,

Miss Lauren Philips

Principal Investigator

Cell: 072 658 7623

Work: 021 938 9258

Email: laurenp@sun.ac.za

ADDENDUM I

Participant information leaflet and consent form

TITLE OF THE RESEARCH PROJECT:

The association between tuberculosis and the development of insulin resistance in adults with pulmonary tuberculosis in the Western Sub-District of the Cape Metropole region

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR: Lauren Philips, Registered Dietician (SA)

ADDRESS: Division of Human Nutrition, Faculty of Medicine and Health Sciences, Stellenbosch University

CONTACT DETAILS: Principal Investigator: 072 658 7623

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the investigator, doctor or nursing sister any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is ***entirely voluntary*** *and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.*

This study has been approved by the ***Health Research Ethics Committee at Stellenbosch University*** *and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.*

WHAT IS THIS RESEARCH STUDY ALL ABOUT?

Participants will be recruited from the Albow Gardens clinic and data collection performed on site at the Brooklyn Chest Hospital, both in the Western Sub-District of the Cape Metropole region. The aim is to include at least 60 participants in the study, of which 30 persons will be chosen to come back

for two (2) follow-up visits. Participants will be signed up for the study over a period of five (5) months and the maximum amount of time you will be involved in the study is five (5) months.

Tuberculosis (TB) is currently a big concern, and is the 2nd leading cause of death worldwide, after HIV/AIDS. The number of TB cases in South Africa, and especially in the Western Cape, is also growing. The aim of the study is to gather valuable information regarding the nature of infection in persons with TB. This aim also includes investigating whether persons with TB have insulin resistance, which occurs when the body doesn't respond normally to insulin (a substance responsible for controlling your body's blood sugar levels).

Basic non-harmful measurements such as weight, height, skinfolds, waist and hip circumference will be performed by the investigator. Other measurements, such as blood samples and blood pressure, will be taken and recorded by the staff at the facility according to a specific schedule. These measurements will not interfere with your normal care at the facility, and you will not be exposed to harmful techniques.

Your confidentiality will be guaranteed at all times, and no personal information (such as names, gender, age, address etc.) will be shared. Unique identification numbers will be used for each participant (based on your folder number at the facility).

WHY HAVE YOU BEEN INVITED TO PARTICIPATE IN THIS STUDY?

A need has been identified for a study to be performed based on the fact that there is relatively little information available at present on the possible relationship between TB and insulin resistance. You have been invited because you are newly diagnosed with TB and are HIV-negative. Your involvement in the study will ensure knowledge is gained on this subject, resulting in improved care and treatment of persons with TB.

WHAT WILL YOUR RESPONSIBILITIES BE?

- It will be expected of you to answer some questions, have some body measurements (weight, height, waist and hip measurements, blood pressure etc.) taken and provide a small amount of blood (1 – 3 teaspoons)
    - Blood samples that will be taken include: glucose, insulin, albumin, CRP, white cell count and lipogram
- It will also be your responsibility to ensure that you return to the facility for follow-ups on the days given to you by the investigator

- Certain selected study participants that are part of the follow-up portion of the study (and are, therefore, required to come back to the clinic) will be compensated for their time and transport costs at each visit to the clinic.

WILL YOU BENEFIT DIRECTLY FROM TAKING PART IN THIS RESEARCH?

No immediate benefits exist for participants, but findings of the study will indirectly contribute to an improved understanding of the course of TB and whether insulin resistance occurs in this group of people. This will hopefully lead to an improved level of patient care, as doctors and other health care workers may be able to provide better levels of treatment to people with TB in the future.

ARE THERE RISKS INVOLVED IN YOUR TAKING PART IN THIS RESEARCH?

There are no risks involved in this study, as methods used by the investigator and study staff are not harmful and shouldn't cause pain. Your privacy and confidentiality will also be strictly protected at all times.

IF YOU DO NOT AGREE TO TAKE PART, WHAT ALTERNATIVES DO YOU HAVE?

If you decide not to take part in the study, it will not affect your treatment at the health facility in any way and it will not be held against you.

WHO WILL HAVE ACCESS TO YOUR MEDICAL RECORDS?

The investigator will have access to the information available in the medical records, and to the results of all measurements performed (weight, height, blood samples etc.). The investigator will only be able to gain access to your medical file and perform measurements once you have given your informed consent and signed this form. If the results of this study are published or used in a thesis (final project), your identity will still remain unknown.

WHAT WILL HAPPEN IN THE UNLIKELY EVENT OF SOME FORM OF INJURY OCCURING AS A DIRECT RESULT OF YOUR TAKING PART IN THIS RESEARCH STUDY?

It is very unlikely that you will suffer any form of injury due to this study, but you may experience some discomfort after providing a blood sample.

WILL YOU BE PAID TO TAKE PART IN THIS STUDY AND ARE THERE ANY COSTS INVOLVED?

You will not be paid directly for your involvement in the study, but will be rewarded for your time and transport costs at scheduled visits to the clinic. No additional costs are expected for the participant.

IS THERE ANYTHING ELSE THAT YOU SHOULD KNOW OR DO?

- You may choose to inform your doctor that you are taking part in a research study
- You may contact the principal investigator, Ms. Philips, at 072 658 7623, if there are any further queries or if any problems/injuries are experienced
- You may contact the Health Research Ethics Committee (HREC) at 021 938 9207 (during working hours) if there are any concerns or complaints that have not been adequately addressed by the study investigator or facility staff
- A need may arise for either sponsors of the study, study monitors/auditors or HREC members to inspect the research records – your confidentiality will still be ensured
- You will be informed if there are any significant changes made to the study and will be asked to sign another consent form
- You will receive a copy of this information and consent form for your own record-keeping

DECLARATION BY PARTICIPANT

By signing below, I ……..…… agree to take part in a research study entitled '*The association between tuberculosis and the development of insulin resistance in adults with pulmonary tuberculosis in the Western Sub-District of the Cape Metropole region'*

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable
- I have had a chance to ask questions and all my questions have been adequately answered
- I understand that taking part in this study is voluntary and I have not been pressurised to take part
- I shall receive no direct payment for participating in this study (other than that of transport costs and refreshments on follow-up days)
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way

- I may be asked to leave the study before it has finished, if the investigator feels it is in my best interests, or if I do not follow the study plan, as agreed to

Signed at (*place*) .....…….. on (*date*) ……...…….. 20.....

....

Signature of participant **Signature of witness**

Thumbprint of participant (if illiterate)

DECLARATION BY INVESTIGATOR

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…….. on (*date*) ……...…….. 20.....

....

Signature of investigator **Signature of witness**

DECLARATION BY INTERPRETER

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*) ……..…….. using the language medium of Afrikaans/isiXhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.

- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…..……. on (*date*) ……...……...

....

Signature of interpreter **Signature of witness**

Deelnemerinligtingsblad en Toestemmingsvorm

TITEL VAN DIE NAVORSINGSPROJEK:

Die verband tussen tuberkulose en die ontwikkeling van insulien weerstandigheid in volwassenes met pulmonêre tuberkulose in die Westelike Sub-Distrik van die Kaapse Metropool area.

VERWYSINGSNOMMER:

HOOFNAVORSER: Lauren Philips, Geregistreerde Dieetkundige (SA)

ADRES: Afdeling Menslike Voeding, Fakulteit Geneeskunde en Gesondheidswetenskappe, Universiteit Stellenbosch

KONTAKNOMMER: Hoofnavorser: 072 658 7623

U word genooi om deel te neem aan 'n navorsingsprojek. Lees asseblief hierdie inligtingsblad op u tyd deur aangesien die 'detail' van die navorsingsprojek daarin verduidelik word. Indien daar enige deel van die navorsingsprojek is wat u nie ten volle verstaan nie, is u welkom om die navorsingspersoneel of dokter daaroor uit te vra. Dit is baie belangrik dat u ten volle moet verstaan wat die navorsingsprojek behels en hoe u daarby betrokke kan wees. U deelname is ook ***volkome vrywillig*** *en dit staan u vry om deelname te weier. U sal op geen wyse hoegenaamd negatief beïnvloed word indien u sou weier om deel te neem nie. U mag ook te eniger tyd aan die navorsingsprojek onttrek, selfs al het u ingestem om deel te neem.*

Hierdie navorsingsprojek is deur die *Gesondheidsnavorsingsetiekkomitee (GNEK) van die Universiteit Stellenbosch* ***goedgekeur en sal uitgevoer word volgens die etiese riglyne en beginsels van die Internasionale Verklaring van Helsinki en die Etiese Riglyne vir Navorsing van die Mediese Navorsingsraad (MNR).***

WAT BEHELS HIERDIE NAVORSINGSPROJEK?

Die studie sal by die Albow Gardens kliniek, asook op die perseel van die Brooklyn Chest Hospitaal plaasvind. Die bogenoemde is albei in die Westelike Sub-Distrik van die Kaapse Metropool area. Daar word beoog om ten minste 60 deelnemers in die studie in te sluit, waarvan 30 individue gekies sal word om terug te kom vir twee (2) opvolg besoeke. Deelnemers sal deel

vorm van die studie vir ’n periode van vyf (5) maande en die maksimum tydsduur wat u deel sal hê aan die studie is vyf (5) maande.

Tuberkulose (TB) is tans ’n groot bron van kommer aangesien dit wêreldwyd die 2de grootste oorsaak van sterfte, naas MIV/VIGS, is. Die hoeveelheid TB gevalle in Suid-Afrika en veral in die Wes-Kaap, is ook aan die toeneem. Die doel van die studie is om waardevolle inligting met betrekking tot die aard van die infeksie in individue met TB in te win. Die doel is ook om vas te stel of mense met TB insulien weerstandig [’n toestand waartydens die liggaam nie normaal teenoor insulien (’n substans wat verantwoordelik is om die liggaam se bloedsuikervlakke te beheer) reageer nie] is?

Basiese nie-skadelike meetings soos gewig, lengte, velvoue en middel- en heup-omtrek sal deur die navorser uitgevoer word. Ander meetings soos die neem van bloedmonsters en bloeddruk sal volgens ’n spesifieke skedule deur die kliniek personeel geneem en aangeteken word. Die meetings sal nie met u gewone sorg by die kliniek inmeng nie en u sal ook nie aan enige skadelike prosedures blood gestel word nie.

Vertroulikheid van u inligting sal te alle tye verseker word en geen persoonlike inligting (soos u naam en van, geslag, ouderdom, adres ens.) sal aan ander uitgegee word nie. Unieke identifikasie nommers (gebaseer op u kliniek lêer nommer) sal vir elke deelnemer gebruik word.

WAAROM IS U GENOOI OM DEEL TE NEEM?

’n Behoefte is geïdentifiseer om ’n studie te loots aangesien daar tans relatief min inligting rakende die moontlike verband tussen TB en insulien weerstandigheid bestaan. U word uitgenooi om deel te neem aan die studie aangesien u nuut gediagnoseer is met TB en MIV-negatief is. U deelname aan die studie sal bydra tot die vermeerdering van kennis rakende die onderwerp, wat in die toekoms sal help met beter sorg en behandeling van mense met TB.

WAT SAL U VERANTWOORDELIKHEDE WEES?

- Daar sal van u verwag word om ’n paar vrae te beantwoord, beskikbaar te wees vir sekere liggaamsmetings (gewig, lengte, middel- en heupomtrek, bloeddruk ens.) asook om ’n klein hoeveelheid bloed (1 – 3 teelepels) te verskaf
    - Bloedmonsters wat geneem gaan word sluit in: glukose, insulien, albumien, CRP, wit sel telling en lipogram

- Dit sal ook u verantwoordelikheid wees om na die kliniek terug te keer (op die spesifieke dae soos deur die navorser versoek) vir u opvolgsessies
- Sekere geselekteerde deelnemers wat ook deel is van die opvolg fase van die projek (en weer na die kliniek toe moet kom) sal weereens vergoed word vir hul tyd en vervoer onkostes tydens elke kliniek besoek.

SAL U VOORDEEL TREK DEUR DEEL TE NEEM AAN HIERDIE NAVORSINGSPROJEK?

Daar is nie enige onmiddellike voordele vir deelnemers nie, maar die studie resultate sal indirek bydra tot ’n beter begrip van die verloop van TB , tesame met die antwoord op of insulien weerstandigheid in die groep mense voorkom of nie. Dit sal hopelik bydra tot ’n beter vlak van pasiëntsorg, aangesien dokters en ander gesondheidswerkers in staat sal wees om beter behandeling aan mense met TB in die toekoms te verskaf.

IS DAAR ENIGE RISIKO'S VERBONDE AAN U DEELNAME AAN HIERDIE NAVORSINGSPROJEK?

Daar is geen risiko’s vir u tydens u deelname aan die studie nie. Alle prosedure wat deur die navorser en kliniek personeel uitgevoer gaan word, is skadeloos en sal nie enige pyn veroorsaak nie. U privaatheid en vertroulikheid van u inligting sal te alle tye verseker word.

WATTER ALTERNATIEWE IS DAAR INDIEN U NIE INSTEM OM DEEL TE NEEM NIE?

Sou u besluit om nie aan die studie deel te neem nie, sal dit nie u sorg by die kliniek enigsins beïnvloed nie. Dit sal ook nie teen u gehou word nie.

WIE SAL TOEGANG HÊ TOT U MEDIESE REKORDS?

Die navorser sal toegang hê tot alle beskikbare inligting in u mediese lêer, asook tot die resultate van alle metings wat op u uitgevoer (gewig, lengte, bloedmonsters ens.) is. Die navorser sal egter slegs toegang tot u mediese rekords ontvang, en meetings op u uitvoer, wanneer u u ingeligte toestemming gee en die vorm teken. Sou die resultate van die projek gepubliseer of gebruik word in ’n tesis (finale projek), sal u identiteit steeds onbekend bly.

WAT SAL GEBEUR IN DIE ONWAARSKYNLIKE GEVAL VAN 'N BESERING WAT MAG VOORKOM AS GEVOLG VAN U DEELNAME AAN HIERDIE NAVORSINGSPROJEK?

Dit is onwaarskynlik dat u enige besering tydens die studie sal opdoen, u mag egter 'n mate van ongemak na afloop van die neem van die bloedmonster ervaar.

SAL U BETAAL WORD VIR DEELNAME AAN DIE NAVORSINGSPROJEK EN IS DAAR ENIGE KOSTE VERBONDE AAN DEELNAME?

U sal nie direkte betaling vir u deelname aan die studie ontvang nie, maar sal vergoed word vir u tyd en vervoer onkostes tydens gereelde kliniek besoeke. Geen verdere onkoste word van u verwag nie.

IS DAAR ENIGIETS ANDERS WAT U MOET WEET OF DOEN?

- U is welkom om u dokter in kennis te stel van u deelname aan die navorsingstudie
- U mag die hoof-navorser, Me. Philips, by 072 658 7623, kontak indien u enige verdere vrae het of indien daar enige probleme/beserings is
- U mag die Gesondheidsnavorsingsetiekkomitee (GNEK) by 021 938 9207 (tydens kantoor ure) kontak sou u enige bekommernis of probleem beleef wat nie toepaslik deur die navorser of kliniek personeel aangespreek word nie
- Dit kan gebeur dat die borge van die studie, studie monitors/ouditeure of GNEK-lede die navorsing rekords moet inspekteur – vertroulikheid van u inligting sal steeds verseker word
- U sal in kennis gestel word indien daar enige beduidende veranderinge aan die studie in die toekoms aangebring word; u sal dan gevra word om nog 'n toestemmingsvorm te teken
- U sal 'n kopie van die toestemmingsvorm vir u eie rekords ontvang

<u>VERKLARING DEUR DEELNEMER</u>

Met die ondertekening van hierdie dokument onderneem ek,
……..……...……..……., om deel te neem aan 'n navorsingsprojek getiteld *'Die verband tussen tuberkulose en die ontwikkeling van insulien weerstandigheid in volwassenes met pulmonêre tuberkulose in die Westelike Sub-Distrik van die Kaapse Metropool area'.*

Ek verklaar dat:

- Ek hierdie inligtings- en toestemmingsvorm gelees het of aan my laat voorlees het en dat dit in ’n taal geskryf is waarin ek vaardig en gemaklik mee is.
- Ek geleentheid gehad het om vrae te stel en dat al my vrae bevredigend beantwoord is.
- Ek verstaan dat deelname aan hierdie navorsingsprojek **vrywillig** is en dat daar geen druk op my geplaas is om deel te neem nie.
- Ek te eniger tyd aan die navorsingsprojek mag onttrek en dat ek nie op enige wyse daardeur benadeel sal word nie.
- Ek gevra mag word om van die navorsingsprojek te onttrek voordat dit afgehandel is indien die studiedokter of navorser van oordeel is dat dit in my beste belang is, of indien ek nie die ooreengekome navorsingsplan volg nie.

Geteken te *(plek)* .....…….. op *(datum)* ……...……. 20.....

..... **.....**

Handtekening van deelnemer Handtekening van getuie

VERKLARING DEUR NAVORSER

Ek *(naam)* ……...……. verklaar dat:

- Ek die inligting in hierdie dokument verduidelik het aan …….....…….
- Ek hom/haar aangemoedig het om vrae te vra en voldoende tyd gebruik het om dit te beantwoord.
- Ek tevrede is dat hy/sy al die aspekte van die navorsingsprojek soos hierbo bespreek, voldoende verstaan.
- Ek ’n tolk gebruik het/nie ’n tolk gebruik het nie. (*Indien ’n tolk gebruik is, moet die tolk die onderstaande verklaring teken.)*

Geteken te *(plek)* .....…….. op *(datum)* ……...……. 20.....

.....	.....
Handtekening van navorser	Handtekening van getuie

VERKLARING DEUR TOLK

Ek *(naam)* ……..…… verklaar dat:

- Ek die navorser *(naam*) …….……. bygestaan het om die inligting in hierdie dokument in Afrikaans/Xhosa aan *(naam van deelnemer)* ……..... te verduidelik.
- Ons hom/haar aangemoedig het om vrae te vra en voldoende tyd gebruik het om dit te beantwoord.
- Ek ’n feitelik korrekte weergawe oorgedra het van wat aan my vertel is.
- Ek tevrede is dat die deelnemer die inhoud van hierdie dokument ten volle verstaan en dat al sy/haar vrae bevredigend beantwoord is.

Geteken te *(plek*) .....…….. op *(datum*) ……...…….. 20…….

.....	.....
Handtekening van tolk	Handtekening van getuie

Incwadana Engolwazi Ngomthathi Nxaxheba Kunye Nefomu Yemvumelwano

ISIHLOKO SEPROJEKTHI YOPHANDO:

Unxulumano phakathi kwesifo sephepha kunye nokuvela kongquzulwano ne-insulini kubantu abadala abanesifo sephepha semiphunga kwiNgingqana yeNtshona yengingqi yeKapa eliMbaxa

INOMBOLO YONXULUMANO:

UMPHANDI OYINTLOKO: Lauren Philips, Incutshe kwisondlo ebhalisiweyo (eMzantsi Afrika)

IDILESI: Icandelo leSondlo saBantu, iFakhalthi yezoNyango neeNzululwazi zeMpilo, eYunivesithi yaseStellenbosch

***IINKCUKACHA ZOQHAGAMSHELWANO**:* Umphandi oyiNtloko: 072 658 7623

Uyamenywa ukuba athathe inxaxheba kwiprojekthi yophando. You are being invited to take part in a research project. Nceda thatha ixesha lokufunda ulwazi oluvezwe apha, oluzakuthi luchaze iinkcukacha zale projekthi. Nceda buza nayiphina imibuzo emalunga nayiphina indawo ongayiqondiyo ngokupheleleyo kubasebenzi besi sifundo okanye kugqirha. Kubaluleke kakhulu ukuba waniliseke ngokupheleleyo yinto yokuba ucacelwe kakuhle ukuba yintoni ebangwa sesi sifundo kwaye ungabandakanyeka njani. Kwakhona, ukuthatha kwakho inxaxheba ***kungentando yakho ngokupheleleyo*** *kwaye ukhululekile ukuba ungarhoxa ekuthatheni inxaxheba. Ukuba uthi hayi, oku akusayi kuchaphazela ukungavumi kwakho nangayiphina indlela. Ukwakhululekile ukuba uyeke kwesi sifundo naninina, nkqu nokokuba uyavuma ukuthatha inxaxheba ekuqaleni.*

Olu phando luvunywe ziinqobo ezisesikweni *zeKomiti yoPhando Lomntu kwiYunivesithi yaseStellenbosch* ***kwaye luzakwenziwa ngokwemigaqo esesikweni lophando elamkelekileyo kwiSaziso sehlabathi sika-Helsinki, iMigaqo eLungileyo yoMzantsi Afrika yokuSebenza eKliniki kunye neBhunga lezoPhando ngamaYeza (MRC) iMigaqo yeNqobo yezoPhando.***

SIMALUNGA NANTONI ESI SIFUNDO SOPHANDO?

Abathathi-nxaxheba baza kufunwa eKlinikhi yase-Albow Gardens, luze uqokelelo lweenkcukacha lwenziwe esiBhedlele sesiFuba iBrooklyn, zombini ezi klinikhi zikwiNgingqana eseNtshona yengingqi yeKapa eliMbaxa. Injongo kukuba kuqukwe ubuncinane abathathi-nxaxheba abangama-60 kolu phando, apho kulo abantu abangama-30 baza kukhethwa ukuze babuye beze kutyelelo lokulandelela lweentsuku ezimbini (2). Abathathi-nxaxheba baza kuba kolu phando ngapha kwexesha leenyanga ezintlanu (5) kwaye elona xesha lininzi eliza kuthathwa kolu phando ziinyanga ezintlanu (5).

Isifo sephepha (i-TB) sixhalabisa kakhulu, kwaye sisizathu sesibini sokufa kwihlabathi liphela, emva kwentsholongwane kagawulayo nogawulayo buqu. Inani labantu abanesifo sephepha eMzantsi Afrika, nangakumbi eNtshona Koloni nalo liyakhula. Injongo yolu phando kukuqokelela ulwazi olululo olumalunga nohlobo lolosuleleko kubantu abanesifo sephepha. Injongo ikwaquka nokuphanda ukuba ingaba abantu abanesifo sephepha banongquzulwano lwe-insulini na, olwenzeka xa umzimba ungaphenduli ngendlela eqhelekileyo kwi-insulini (into enoxanduva lokulawula amanqanaba akho eswekile yegazi lomzimba).

Imilinganiselo esisiseko engenangozi enje ngobunzima, ubude, ukusongana kolusu,kunye nesazinge sesinqe nesenyonga iza kwenziwa ngumphandi. Eminye imilinganiselo enje ngamasampula egazi kunye noxinzelelo lwegazi iza kuthathwa ize ibhalwe ngabasebenzi abaseklinikhi ngokwesicwangciso esithile. Le milinganiselo ayisayi kuphazamisana nonakekelo lwakho lwesiqhelo eklinikhi, kwaye akusayi kuvezeka kwizenzo eziyingozi.

Ukuba yimfihlo kwakho kuza kuqinisekiswa ngamaxesha onke, kwaye akukho lwazi lwakho (olunje ngamagama, isini, ubudala, idilesi, njalo njalo) kuza kwabelwana ngalo. Kuza kusetyenziswa iinombolo zesazisi ezifana zodwa kumthathi-nxaxheba ngamnye (ezisekelwe kwinombolo yakho yefowulda yaseklinikhi).

KUTHENI UMENYIWE UKUBA UTHATHE INXAXHEBA?

Kuye kwabonwa imfuneko yokuba kwenziwe uphando kusukelwa kwinto yokuba kukho ulwazana oluncinane olukhoyo olumalunga nonxulumano olunokuba khona phakathi kwesifo sephepha (TB) kunye nongquzulwano lwe-insulini. Umenywe kuba kusanda kufunyaniswa ukuba unesifo sephepha, kwaye ukuthatha kwakho inxaxheba kolu phando kuza kuqinisekisa ukuba

kufunyanwa ulwazi kwesi sifundo, loo nto ikhokelele kunakekelo nonyango oluphuculweyo lwabantu abanesifo sephepha.

LUYAKUBA YINTONI UXANDUVA LWAKHO?

- Kuza kulindelwa ukuba uphendule eminye imibuzo, kuthathwe imilinganiselo yomzimba (ubunzima, ubude, nemilinganiselo yesinqe neyenyonga, uxinzelelo lwegazi, njalo njalo) uze unikeze nentwanana yegazi (1 –3 amatispuni)
    - Amagazi azotsalwa ngala: iSwekile, insulini, iAlbumin, iCPR, iWhite cell count, iLipogram
- Iza kuba luxanduva lwakho ukuqinisekisa ukuba ubuyela eklinikhi ukuza kutyelelo lolandelelo ngeentsuku ozinikwe ngumphandi
- Abantu abakhethiweyo abazothatha uxanduva kuyanyanzeleka babuyele eklinikhi bazobonelelwa ngexesha kabo nange mali yokhwela quo xabe vakashela eklinikhi

INGABA UZA KUZUZA EKUTHATHENI INXAXHEBA KOLU PHANDO?

Akukho zibonelelo zikhawulezileyo kubathathi-nxaxheba, kodwa imiba efunyenwe kolu phando iza kuba negalelo elingathanga ngqo ekuqondeni kakuhle inkqubo yesifo sephepha, kwanokuba ingaba ungquzulwano lwe-insulini luyenzeka na kweli qela labantu. Kuthenjwa ukuba le nto iza kukhokelela kwinqanaba eliphuculweyo lonakekelo lwesigulana, njengoko oogqirha kunye nabanye abasebenzi bonakekelo lwempilo benokwazi ukunikeza amanqanaba angcono onyango kubantu abanesifo sephepha kwixa elizayo.

INGABA ZIKHO IINGOZI EZIBANDAKANYEKAYO EKUTHATHENI KWAKHO INXAXHEBA KOLU PHANDO?

Akukho mingcipheko ikhoyo kolu phando, njengoko izenzo ezisetyenziswa ngumphandi kunye nabasebenzi bolu phando zingenangozi kwaye zingasayi kwenza iintlungu uzothi ke ubonelelwe rhoqo xa uze eklinikhi koluphando. Ukuba bucala kunye nokuba yimfihlo kwakho kuza kukhuselwa ngendlela engqongqo amaxesha onke.

UKUBA AWUVUMI UKUTHATHA INXAXHEBA, LOLUPHI OLUNYE UNYANGO ONALO?

Ukuba ugqiba kwelokuba ungathathi nxaxheba kolu phando, loo nto ayisayi kuchaphazela unyango lwakho eklinikhi nangaluphina uhlobo, kwaye loo nto ayisayi kukubalela.

NGUBANI UZA KUFUMANA INGXELO YAKHO YAMAYEZA?

Umphandi uza kufikelela kulwazi olukhoyo kwiingxelo zonyango, kunye nakwiziphumo zayo yonke imilinganiselo eyenziweyo (ubunzima, ubude, amasampula egazi, njalo njalo). Umphandi uza kufikelela kuphela kwifayile yakho yonyango aze enze imilinganiselo xa unike imvume eyiyo waze watyikitya le fomu. Ukuba iziphumo zolu phando ziyashicilelwa okanye zisetyenziswa kwithisisi (umsebenzi wokugqibela), ukwaziwa kwakho kuza kuhlala kungaziwa.

KUZA KWENZEKA NTONI KWIMEKO YESIGANEKO ESINGALINDEKANGA SOKWENZAKALA NGENXA YOKUTHATHA KWAKHO INXAXHEBA KWESI SIFUNDO SOPHANDO?

Kuthandabuzeka kakhulu ukuba uza kufumana naluphina uhlobo lomonzakalo ngenxa yolu phando, kodwa usenokufumana ukungonwabi emva kokunikezela ngesampula legazi. .

INGABA UZA KUHLAWULWA NGOKUTHATHA INXAXHEBA KWESI SIFUNDO KWAYE INGABA KUKHO IINDLEKO EZIBANDAKANYEKAYO?

Akusayi kuhlawulwa ngokuthe ngqo ngokuthatha kwakho inxaxheba kolu phando, kodwa uza kubonelelwa ngexesha lakho nangeendleko zesithuthi. Akulindelwanga ezinye iindleko ngokuthatha inxaxheba.

INGABA IKHO ENYE INTO EKUMELE UYAZI OKANYE UYENZE?

- Ungakhetha ukuxelela ugqirha wakho ukuba uthatha inxaxheba kwisifundo sophando.
- **Ungaqhgamshelana noGqr kule inombolo yomnxeba ukuba unemibuzo engaphaya okanye uhlangabezana neengxaki.**
- **Ungaqhagamshelana neKomiti yoPhando Lomntu kwa-021-938 9207 ukuba unenkxalabo okanye izikhalazo ezingasonjululwanga kakuhle ngugqirha wakho wesifundo.**
- Kusenokubakho imfuneko yokuba abaxhasi bolu phando, abalawuli/abaphicothi bolu phando okanye amalungu e-HREC ahlole iingxelo zolu phando – nalapho ukuba yimfihlo kwakho kuza kuqinisekiswa
- Uza kwaziswa ukuba kukho utshintsho olukhulu olwenziwayo kolu phando kwaye uza kucelwa ukuba utyikitye enye ifomu yemvume
- Uza kufumana ikopi yolu lwazi kunye neyefomu yemvume ukuze uzigcinele zona

ISIFUNGO SOMTHATHI-NXAXHEBA

Ngokuytyikitya ngezantsi, Mna ……..……. ndiyavuma ukuthatha inxaxheba kwisifundo sophando semfuzo esibizwa ngokuba *(faka ishloko sesifundo)*.

Ndazisa ukuba:

- Ndilufundile okanye ndalufunda olu lwazi kunye nefomu yemvumelwano kwaye ibhalwe ngolwimi endiliciko nendikhululekileyo kulo
- Bendinalo ithuba lokuba ndibuze imibuzo kwaye yonke imibuzo yam iphendulwe ngokwanelisayo.
- Ndiyakuqonda ukuba ukuthatha inxaxheba kolu phando kube **kukuzithandela kwam** kwaye andikhange ndinyanzelwe ukuba ndithathe inxaxheba.
- Ndingakhetha ukusishiya isifundo naninina kwaye andisayi kohlwaywa okanye uqal' ugwetywe nangayiphi indlela.
- Usenokucelwa ukuba usishiye isifundo phambi kokuba siphele, ukuba ugqirha wesifundo okanye umphandi ukubona kuyinzuzo kuwe, okanye ukuba andisilandeli isicwangciso sesifundo, ekuvunyelenwe ngaso.

Kutyikitywe e-(indawo) .....…..……... ngo-(usuku) ……...…….. 20……..

..... **.....**

Umtyikityo womthathi-nxaxheba **Umtyikityo wengqina**

ISIFUNGO SOMPHANDI

Mna *(igama)* …… ndiyafunga ukuba:

- Ndilucacisile ulwazi olu kweli xwebhu ku-……..……...
- Ndimkhuthazile ukuba abuze imibuzo kwaye athathe ixesha elifanelekileyo ukuba ayiphendule.
- Ndiyaneliseka kukuba uyakuqonda ngokwanelisayo konke okumalunga nophando okuxoxwe ngasentla.

- Ndisebenzise/andisebenzisanga toliki. (*Ukuba itoliki isetyenzisiwe kumele ityikitye isaziso ngezantsi.*

Kutyikitywe e-(indawo) .....…..…….. ngo-(usuku) ……...…….. 20.....

..... **.....**

Umtyikityo womphandi Umtyikityo wengqina

ISIFUNGO SETOLIKI

Mna *(igama)* …… ndazisa ukuba:

- Ndicende umphandi (*igama*) ……. Ekucaciseni ulwazi olu lapha kweli xwebhu ku-(*igama lomthathi-nxaxheba*) …….. ndisebenzisa ulwimi lwesiAfrikaans/lwesiXhosa.
- Simkhuthazile ukuba abuze imibuzo kwaye athathe ixesha elifanelekileyo ukuba ayiphendule.
- Ndimxelele eyona nto iyiyo malunga nokunxulumene nam.
- Ndiyaneliseka kukuba umthathinkxaxheba ukuqonda ngokupheleleyo okuqulathwe loluxwebhu lwemvumelwano eyazisiweyo kwaye nemibuzo yakhe yonke iphendulwe ngokwanelisayo.

Kutyikitywe e-(*indawo*) .....…..…….. ngo-(*usuku*) ……...…….. 20.....

..... **.....**

Umtyikityo wetoliki Umtyikityo wengqina